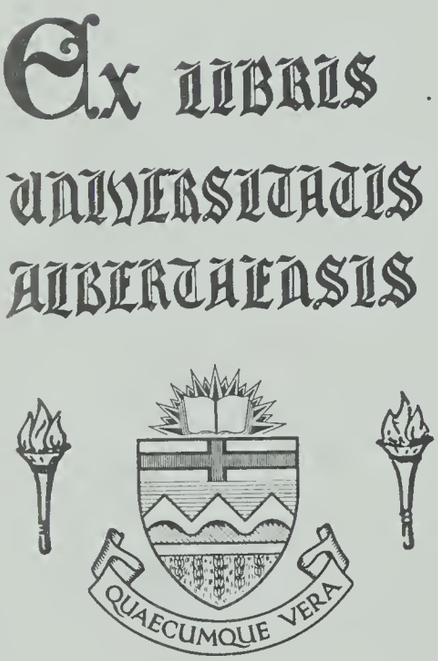


The University of Alberta
Printing Department
Edmonton, Alberta

THE UNIVERSITY OF ALBERTA

HYDROLYSIS OF POLYPHOSPHATES ADDED TO MEAT

by

© MOHAMED K. AWAD, B.SC.

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA

OCTOBER, 1968

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

HYDROLYSIS OF POLYPHOSPHATES

ADDED TO MEAT

submitted by Mohamed K. Awad in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

Investigations conducted during the past two decades indicate that the use of polyphosphates in meat processing is widely practices. The polyphosphates increase water-holding capacity, improve emulsification, stablize fat, improve flavor and eating quality of the products. There is evidence that color - and color retention - are improved.

The fate of added inorganic polyphosphates to meat, and their effect on pH and the soluble nitrogen content, has been investigated.

The polyphosphates added were significantly hydrolyzed to the ortho form in both of fresh and heat pretreated meat. The pH values of phosphate-treated meat samples were generally different from the untreated samples.

A small variation in soluble nitrogen was observed in most heat-treated samples, while larger changes in soluble nitrogen occurred in the case of some fresh meat samples. In other fresh meat samples soluble nitrogen values remained constant.

It has been concluded that enzyme systems are present in fresh meat, which decrease the effectiveness of polyphosphate addition. These enzyme systems can be effectively deactivated by mild heat treatment.

ACKNOWLEDGMENT

The author wishes to express his appreciation to his supervisor, Dr. J.M. deMan, under whose help and guidance this investigation was conducted and wishes to thank him for his constant advice and encouragement.

The author wishes to express his sincere appreciation to Dr. F.H. Wolfe for his assistance in the reviewing of this manuscript.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

INTRODUCTION

The first recorded use of phosphates in foods - and their major use for many years - was as an ingredient of baking powder a century or more ago. At that time very little was known about how phosphates bring about improvements in food quality. World War II stimulated new techniques in food processing, and these led to new types of food products. Since that time, manufacturers and their chemists developed new methods of compounding and stabilizing foods so that products would posses longer shelf life, and broader tolerance in cooking, both for the housewife as well as for commerical use.

Phosphates have contributed in many ways to these new techniques of processing. While the literature concerning food additives - including phosphates - is very extensive, very little work has been published so far about the fate of added condensed phosphates and their interaction with food constituents.

The object of the present investigation is concerned with the stability of added condensed phosphates in meats.

General Principles

As a first consideration this study requires a knowledge of both the characteristics of the meat and the chemistry of phosphates.

Meat is primarily composed of lean muscle and connective tissue, this being made up of approximately 75% water and 18% protein, the remaining 7% consisting of fat, carbohydrates, minerals and non-protein

water extractives.

1. The water of meat:

Muscle, like other biological material, contains water of hydration (electrostatically bound water) and physically absorbed water, held on the proteins by the secondary forces such as water dipole - dipole induction, hydrogen bonds, and capillary and surface attractions.

2. The proteins of meat:

Proteins are second only to water as the most abundant substance in most animal tissues and are, without doubt, the most important constituents of the edible portion of meat animals. The most abundant muscle protein is the globulin complex actomyosin,which is responsible for the contractile properties of muscle. This complex actually consists of two proteins, actin and myosin. Edible muscle tissue also contains lesser quantities of:

a. Collagen, reticulin, and elastin, whose only known function is to provide structure and means of attachment to the skeleton;

b. the respiratory pigment myoglobin, which is responsible for the color of meat;

c. nucleoproteins, which in the living cell are a major constituent of the genetic material that controls the heritable characteristics of the cell;

d. enzymes, which function as catalysts for virtually every metabolic reaction that takes place within the

living cell; and

e. an array of other protein components with miscellaneous functions.

3. The inorganic constituents of meat:

The inorganic constituents in muscle cells are the same as those in most cells of the body. Potassium is the most common cation, with magnesium and sodium. The anions are phosphate, bicarbonate, and sulfate. The salts in the extracellular fluid have a concentration equal to that in the blood plasma. Sodium is the most important cation with potassium, magnesium, and calcium in small amounts. The dominant anion is chloride, with appreciable amounts of bicarbonate and small amounts of phosphate and sulfate.

The phosphates:

By definition, the phosphates are those compounds of phosphorus in the anions of which each atom of phosphorus is surrounded by four oxygen atoms arranged to corners of a tetrahedron. By sharing oxygen atoms between tetrahedra, chains, rings, and branched polymers of interconnected PO_4 tetrahedra can be produced.

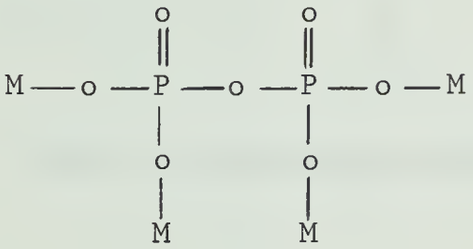
Combinations of PO_4 groups:

In the case of a single PO_4 group, there is just one arrangement, that of the orthophosphate molecule as shown here:

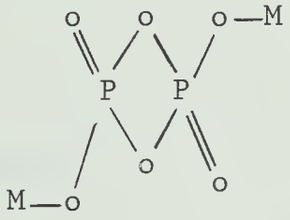
orthophosphate, M_3PO_4

Since the orthophosphate molecule is the only example in the family of phosphates of a single PO_4 group, and the condensed phosphates are made up of interconnected PO_4 groups, it is apparent that the term "condensed phosphates" is very fitting for these compounds.

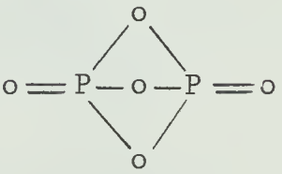
In the case of two PO_4 groups, there are three possible arrangements, these are depicted below:



Pyrophosphate, $M_4P_2O_7$



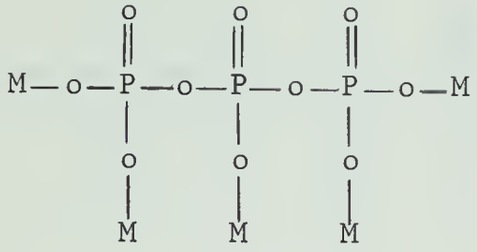
Dimetaphosphate, $(MPO_3)_2$



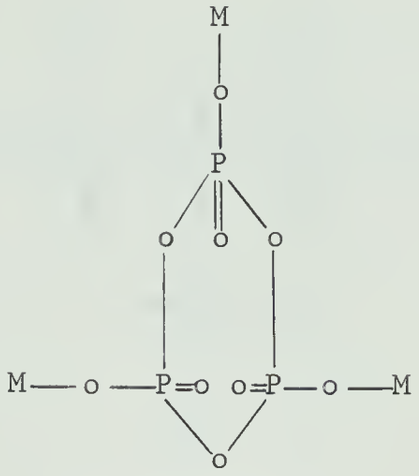
Monomeric phosphorus pentoxide

Only one of these three, the pyrophosphate, is known. The other two structures involve considerable strain and have not been proven to exist.

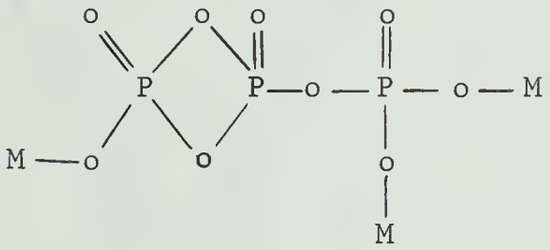
There are four ways in which three PO_4 groups can be connected. These four structures are shown below:



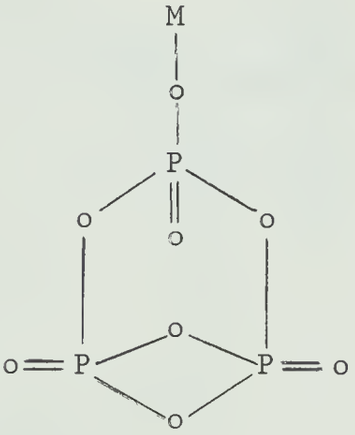
tripolyphosphate, $M_5P_3O_{10}$



trimetaphosphate $(MPO_3)_3$



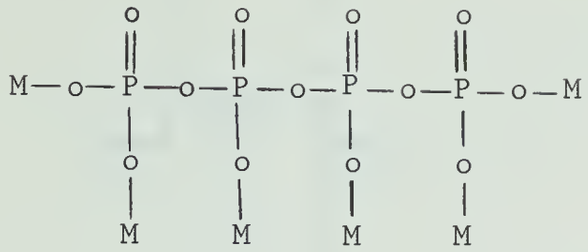
"ISO" trimetaphosphate $(MPO_3)_3$



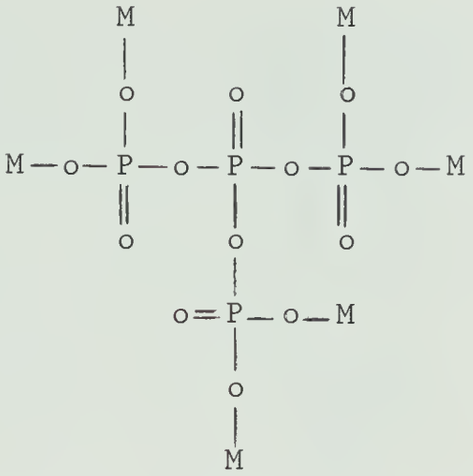
An ultraphosphate, MP_3O_8

The linear structure, called the tripolyphosphate, is well known, and so is the simple ring structure called the trimetaphosphate. The other two structures, which involve dimetaphosphate bridges, have not been shown to exist, probably, because of the large strain which occurs in the dimetaphosphate bridge.

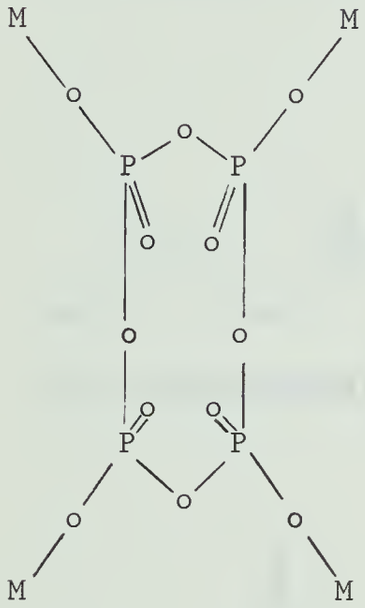
There are eleven different ways in which four groups can be put together, as shown below:



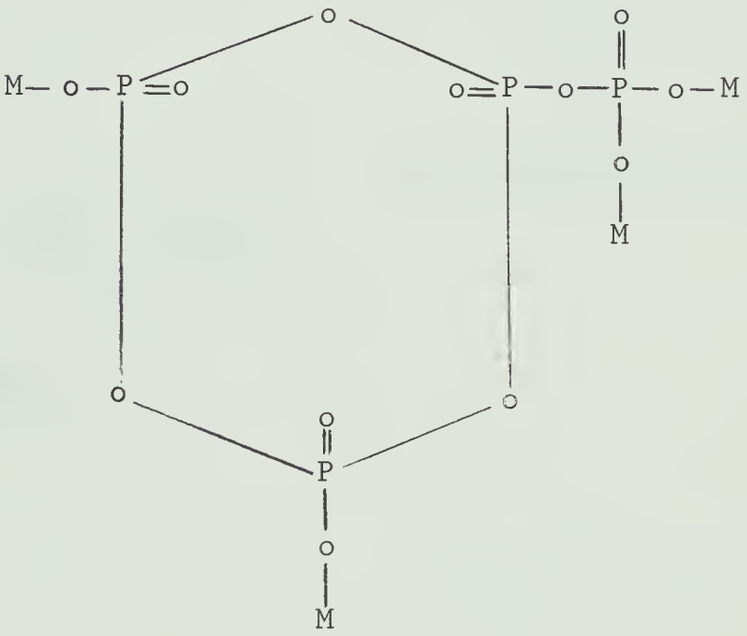
Tetrapolyphosphate, $M_6P_4O_{13}$



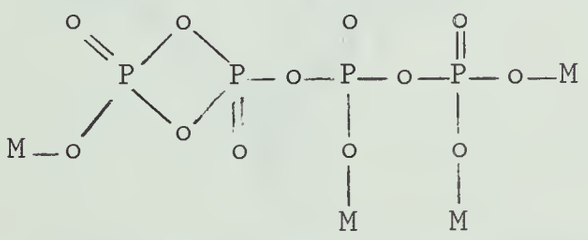
"Iso" tetrapolyphosphate, $M_6P_4O_{13}$



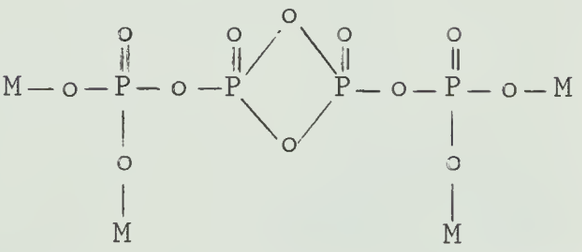
Tetrametaphosphate, $(MPO_3)_4$



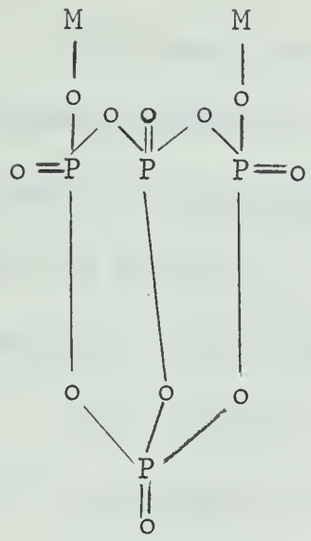
"Iso" tetrametaphosphate, $(MPO_3)_4$



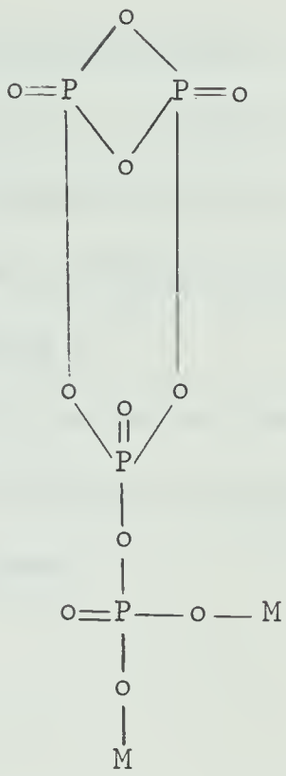
"Iso" tetrametaphosphate, $(MPO_3)_4$



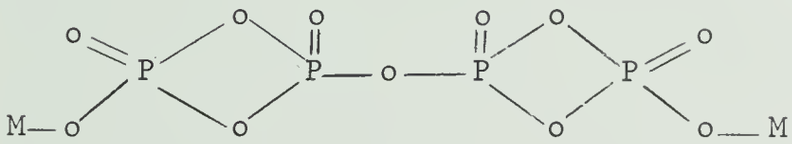
"Iso" tetrametaphosphate, $(MPO_3)_4$



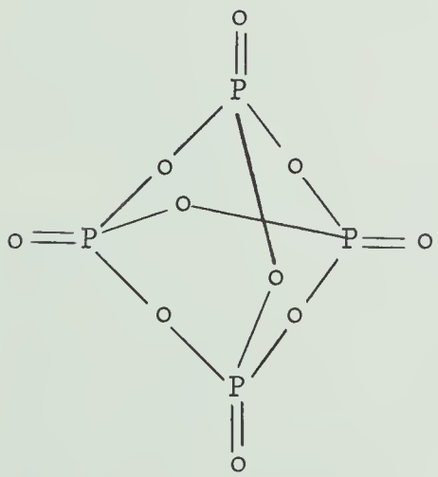
A. An ultraphosphate, $M_2P_4O_{11}$



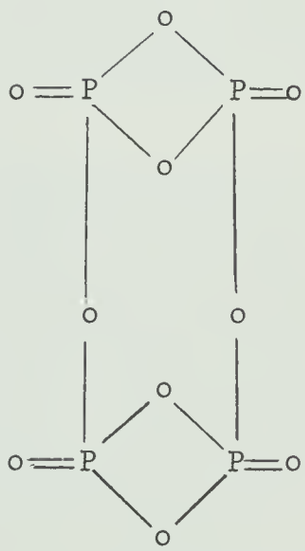
An ultraphosphate, $M_2P_4O_{11}$



An ultraphosphate, $M_2P_4O_{11}$



B. Dimeric phosphorus pentoxide $(P_2O_5)_2$



An isomer of dimeric phosphorus pentoxide $(P_2O_5)_2$

Of these, only three structures - the tetrapolyphosphate, the tetrametaphosphate, and dimeric phosphorus pentoxide - have been shown to exist in crystalline form. Also the ultraphosphate structure, labelled compound A, must have at least a momentary existence in the hydrolysis of the P_4O_{10} molecule - shown as compound B.

This system of building up phosphate structures can be continued, but it is apparent that the number of possible structures increases very rapidly as the number of PO_4 groups is increased.

REVIEW OF LITERATURE

I. The effect of the addition of phosphates and polyphosphates to different foods:

The addition of chain phosphates to meats lowers the quantity of juices exuded during cooking or smoking. Both orthophosphates and condensed phosphates are employed in this application.

Hamm (1955) determined the action of small amounts of phosphates added to meat containing salt, by measuring the pH-increasing effect and the "salt effect". Grau (1955) defended the use of phosphates in comminuted meats. He found that the water retention of meat is dependent upon pH and mineral content, and stated that at a high pH, meat has good water retention, and that this retention drops with decreasing pH and is at it lowest value at pH 5.5. In addition Hamm and Grau (1955) pointed out that the addition of phosphates exerts an effect on water binding, and on the texture of meat. In the presence of salt, an increase in pH produced a greater effect on water binding than in the absence of salt.

Bendall (1954, 1958) observed that by adding solutions of phosphates to raw rabbit meat an increase in the volume of the meat resulted. This increase was as high as 10-20% when a 0.5% phosphate solution was used. Results were similar with 1% sodium chloride. However, when these two compounds were added together - 0.5% phosphate and 1% sodium chloride - there was a 30% increase in volume of the raw meat when orthophosphate was used and at least 55% when disodium dihydrogen pyrophosphate

was used. Phosphate treated samples decreased to about 65-70% of their original weight during cooking. Combinations of the two chemicals resulted in retention of 20% of the original weight.

Bendall explained the effect of phosphates on water holding capacity by suggesting that the protein actomyosin is broken into its component parts and thereby loses its gel character. Swift and Ellis (1956) reported that control of temperature (32-41°F) was important in obtaining maximum moisture retention in phosphate - treated ground beef and pork. They agreed with Bendall (1954, 1958) that dissolution of the proteins, especially actomyosin, is a major factor influencing water retention of meat treated with phosphates. Other factors are ionic strength and pH. Morse (1955) who reviewed previous work on red meats, noted that pH adjustment to about 7.0-7.4 enables meat protein to hold the normal water content, on the other hand, meat that approaches its iso-electric point - pH 5.5 - loses water and water solubles. The use of a phosphate salt producing pH 7.0 is therefore helpful. Tims and Watts (1958) also showed that pyro-, tripoly-, hexameta-, and orthophosphate decreased cooking losses of meats.

Tanikawa *et al*. (1963) noted that drip formation in cod tissue pretreated with polyphosphates was lowered, and that the amount of actomyosin decreased during storage. Love and Abel (1966) showed that the treatment of frozen cod with polyphosphates reduced the thaw drip, as a result of dissolving protein, with resulting deposition on the surface of treated fillets. The conclusion is in agreement with

previous reports by Bendall (1954, 1958) and Swift and Ellis (1956).

A natural extension of established uses of phosphates and polyphosphates in food processing was the addition of polyphosphates to the chilling water for ready-to-cook poultry, or the addition of polyphosphates in subsequent steps of the manufacture of poultry meat into pre-cooked products. Qualities that have been suggested as benefiting from polyphosphate addition include flavour, flavour stability, tenderness, juiciness, and cooking shrinkage.

May et al. (1962) reported that polyphosphates at 4 oz. per gallon of chilling solution produced greater moisture uptake than water controls, while 8 oz. and 10 oz. per gallon produced less uptake than controls. They reported also that polyphosphates reduced weight loss of cut-up fryers during storage at 35°F, improved juiciness and tenderness of light and dark meat, and flavour of dark meat. This was in agreement with results of Mountney and Arganosa (1962) and Schermerhorn et al. (1963) who found that there was less moisture uptake and less cooking shrink in phosphate-treated birds.

Spencer and Smith(1962) obtained a 1-2 day increase in refrigerated shelf life from a 6-hour chilling period in water containing 10 oz. of polyphosphates per gallon. Results showed also that the polyphosphate treatment resulted in greater tenderness and juiciness but no significant difference in moisture uptake. Similar results were reported by Klose et al. (1963) and Monk et al. (1964), who found that polyphosphates increased the moisture retention in cooked meat

and reduced cooking losses. Froning (1966) found that polyphosphates increased the tear strength when added to ground chicken meat. Colour, texture, and flavour of polyphosphate treated meat were found to be acceptable.

Pre-cooked frozen chicken meat, like most cooked meats, develops off-odor through oxidative deterioration. Off-odor may develop very rapidly, often within a short time after cooking. Lehmann and Watts (1951) demonstrated the effectiveness of a number of condensed phosphates in preventing liquid oxidation in aqueous fat systems. Tims and Watts (1958) reported that pyro-, tripoly-, and hexametaphosphate had an antioxidant effect, whereas orthophosphate did not. Marione and Forsythe (1962) investigated the protection of uncooked turkey meat lipids by tripolyphosphate as well as other substances, and found that tripolyphosphate had significant antioxidant properties. Ramsey and Watts (1963) reported that polyphosphates had a marked effectiveness in inhibiting lipid oxidation of beef and ground mullet (fish). Thomson (1964) agreed with the previous reports, and showed that a phosphate mixture - sodium pyro-, and tripolyphosphate - was effective in inhibiting oxidative deterioration during commercial production of frozen cooked chicken. He reported that throughout one week at 40°F,phosphate-treated chicken meat showed no, or very slight, off-odor and a 2-thiobarbituric acid (TBA) value of about one, whereas untreated chicken had a strong to medium-strong off-odor and a TBA value of about six.

Gisske (1958) reported that phosphates give more intense and stable colour, and increased shelf life of cooked ham. Trumic and

Masic (1962) noted that the use of polyphosphates reduced shrinkage in canned ham during curing as well as during pasteurization.

Phosphates and polyphosphates have been added to milk and milk products, because of their effect on the stability of the products. When normal concentrated milk is heated to the temperatures used for preservation - usually 240°F - it tends to coagulate. Furthermore, this coagulation can take place slowly so that canned milk may set during storage to a cheeselike consistency. Addition of phosphates or citrates prevent coagulation and allow adequate heat processing. Leviton and Pallansch (1962) found that several types of compounds, when added to concentrated milk before sterilization, were beneficial in prolonging storage life. Among the most effective of these additives were polyphosphates. Wilson et al. (1963) noted that when polyphosphates were added to concentrated milk sterilized at 303± 2°F gelation was delayed, whereas orthophosphates enhanced gelation. deMan (1966) found that the addition of orthophosphates to skimmilk slightly reduced the stability as measured by rennet coagulation time, while all polyphosphates tested increased the stability greatly. Furthermore he noted that at polyphosphate concentrations of 40 mg/100 ml or over, the milk did not coagulate at all.

A large number of these applications are based on the sequestering ability of the chain phosphates. Metal ions occur naturally in most food or are absorbed from processing water, or even from processing equipment. Calcium, magnesium, copper, and iron are examples. McFee et al.

(1953) reported that the use of sodium hexametaphosphate at a level of 2% by weight of the solids reduced the formation of struvite crystals in canned fish products. The struvite crystals, magnesium ammonium phosphate hexahydrate, which occasionally form in canned fish,are undesirable because they resemble glass particles and lead to unwanted grittiness. Siewart and Woodrof (1952) used sodium tetraphosphate to assist the penetration of salt brine through peanut shells prior to roasting, thus giving unshelled salted peanuts. Holmquist et al. (1948) added hexametaphosphate during the blanching of peas to prevent adsorption of calcium and magnesium ions from the hard water and this resulted in improved tenderness of the peas.

II. Hydrolysis of condensed phosphates:

Kiehl and Coats (1927) showed that sodium pyrophosphate in aqueous or alkaline solution on long standing at room temperature or even at the boiling point does not change to orthophosphate. In another study Kiehl and Hansen (1926) showed that in acid solution, this change does take place, and its rate is dependent upon the concentration of both pyrophosphate and acid, as well as temperature. However, it has been shown that tripolyphosphate and other condensed phosphates are hydrolyzed to orthophosphate. Van Wazer et al. (1952, 1955) and Crowther and Westman (1954) showed that condensed phosphates hydrolyze in aqueous solutions to yield less condensed phosphates and ultimately pure orthophosphate. The results indicate that the rate of hydrolysis is dependent upon the temperature, pH, concentration of

phosphate, and ionic environment. Van Wazer (1958) summarized the major environmental factors which effect the rate of hydrolysis of condensed phosphates, in what is believed to be their decreasing order of effectiveness. A brief summary of these factors is presented in Table 1.

Table 1. Factors affecting the rate of hydrolysis of condensed phosphates

Factor	Approximate effect on rate
A - Temperature	10^5-10^6 faster from freezing to boiling
B - pH	10^3-10^4 slower from strong acid to base
C - Enzymes	As much as 10^5-10^6 faster
D - Colloidal gel	As much as 10^4-10^5 faster
E - Complexing cations	Very many-fold faster in most cases
F - Concentration	Roughly proportional
G - Ionic environment in the solution	Several - fold faster

A - Temperature:

Van Wazer and Holst (1950) showed that in solutions of any pH at room temperature, or below, the chain and unbranched ring phosphates can be kept for several days without noticeable degradation. Bell (1947) studied the hydrolysis of dehydrated sodium phosphates, and found that temperature greatly affected the rate of hydrolysis, which was much slower at 70° than at 100°C. Campbell and Kilpatrick (1954), Friess (1952), McCullough et al. (1956), Bell (1952), Green (1950), and

Van Wazer et al. (1955) studied the effect of temperature on the rate of hydrolysis of condensed phosphates. In general they found that the activation energy for splitting P - O - P linkages in phosphate chains and rings was 20 - 40 Kcal/mole; the most frequently quoted value was 25 Kcal/mole.

B - pH:

Green (1950) pointed out that the stability of seven dehydrated phosphates decreased with decreasing pH. Crowther and Westman (1954, 1958), McCullough et al. (1958), McGilvery and Crowther (1954), and Bell (1947, 1952) reported that the hydrolytic degradation of all chain and ring phosphates was strongly catalyzed by hydrogen ions, the rates of hydrolysis in acidic solutions were higher than in neutral solutions. Only the hydrolysis of tripolyphosphate was base catalyzed. Tripolyphosphate solutions were found to be most stable in the pH 4.9 - 10 range. Van Wazer et al. (1952) suggested that this apparent base catalysis of tripolyphosphate hydrolysis is due to the formation of complexes with sodium ions.

C - Enzymes:

Using various species of the plant kingdom Karl-Kroupa et al. (1957) studied the hydrolysis of sodium tripolyphosphate in various cellular suspensions, and noted that all of the many varieties of cellular material tested accelerated the rate of hydrolytic breakdown of tripolyphosphate. No catalytic degradation was observed in similar experiments after addition of Kaolin, if the powdered Kaolin was

pretreated by drying in a 110°C oven. They concluded that this is an enzymatic catalysis occurring with a large number of cellular materials. These results are in agreement with those of Sawyer (1952), Englebrecht and Morgan (1959), Cleoceri and Lee (1965a), and Scharpf and Kichline (1967) who reported that the presence of organisms in solutions to which condensed phosphates were added caused an accelerated rate of hydrolysis of these phosphates. Harold and Harold (1965), showed that at least four enzyme systems have been isolated from microbial cells that degrade inorganic polyphosphates.

D - Colloidal gels:

Bamann and Meisenheimer (1938) found that the effect of colloidal gels, such as hydrated oxides of iron, cobalt, nickel, aluminum, and the rare earths, notably accelerated the hydrolytic degradation of chain and ring phosphates.

E - Complexing cations:

Green (1950) reported that calcium usually increases the rate of hydrolysis of condensed phosphates, while in the presence of magnesium the rate of reversion was unaltered or decreased. Although Cleoceri and Lee (1965b) attributed the increased rates of hydrolysis of pyrophosphate and tripolyphosphate to dissolved substances in solution, they found that the hydrolysis rate was more rapid in sterile lake water, which has the highest concentration of calcium ions.

F - Concentration:

Van Wazer et al. (1952, 1955) and Crowther and Westman (1954) indicated that the rate of hydrolysis of condensed phosphates is first

order at constant hydrogen ion concentration, and the rate is dependent upon the temperature, pH, ionic environment, and concentration of phosphates. Furthermore Crowther and Westman (1954) and Smith (1959) found that the hydrolysis of different condensed phosphates in the same dilute solution proceeded independently of each other.

G - Ionic environment in solution:

It is difficult to separate the effect of ionic environment from other factors, especially the effect of complexing cations. Van Wazer et al. (1952, 1955) used tetramethyl-ammonium ion as the swamping electrolyte and found that this ionic environment decreased the rate of hydrolysis in both acidic and basic solutions, and therefore acted according to the Bronsted-Bjerrum rule (Bronsted-Bjerrum, in Van Wazer et al., 1955).

Some work has been done on the fate of added phosphates in food. Roesler (1966) showed that when a polyphosphate emulsifying agent was used in cheese,breakdown occurred during emulsification to orthophosphate. It was found in processed cheese blocks, which have a high solids content, the polyphosphates were broken down to ortho and pyrophosphate, whereas in processed cheese of a low solids content the condensed phosphates were broken down to orthophosphate. He noted that breakdown of the phosphates took place during emulsifying and in the first few days after manufacture independent of the solids content of the cheese. Scharph and Kichline (1967) studied the stability of long chain polyphosphates in aqueous cheese extracts, they found that long

chain species decreased while the orthophosphate and trimeta compound increased, and a considerable microbial growth was observed in the extracts at 20°C. It was pointed out that the growth of the micro-organisms in the 20°C extract undoubtedly catalyzed the breakdown of the phosphates.

III. Interactions between polyphosphates and food constituents:

Strong interactions between polyphosphates and proteins have been found to occur. Herrmann and Perlmann (1937) have reported that egg albumin is precipitated by metaphosphoric acid, and that the maximum number of phosphorus atoms bound by egg albumin molecules is very nearly equal to the number of positively charged groups present in the protein molecule. Perlmann and Herrmann (1938) stated that both the amount of precipitate and the phosphorus content of the precipitate increased to a maximum with increasing amounts of added metaphosphoric acid. Briggs (1940) pointed out that the metaphosphate - protein reaction can be regarded as a complex in which the negative multivalent metaphosphate ion is linked to the positive (amino) groups of the protein by a salt-like bond of very low ionizing capacity. He showed also that no similar reaction occurs between metaphosphate and amino acids or other low molecular weight substances containing single basic groups. Yasui et al. (1964) studied the effects of three inorganic polyphosphates on the solubility of myosin B, natural actomyosin, and on the extractability of structural protein from myofibrils in various conditions. There were two types of effect, the first involved

polyphosphates of comparatively low molecular weight, such as pyrophosphate or tripolyphosphate, which react with salt-free myosin B as a salt. Their affinity to myosin B was greatly improved in the presence of high concentrations of salt and divalent cations. The second type involved highly polymerized polyphosphates such as hexametaphosphate, these were bound directly to salt-free myosin B, but their binding is somewhat inhibited by the presence of high concentrations of salt and divalent cations. A similar conclusion was reached by Lyons and Siebenthal (1966) who studied the effect of chain length on the interaction of proteins with condensed phosphates. It was assumed in their study that any interaction between a condensed phosphate and a protein molecule involves the same sites on the phosphate as are engaged in the formation of soluble calcium complexes with the phosphate. Thus any reduction in sequestering power in the presence of protein may be attributed to protein-phosphate interactions. They showed that there is a mass action effect, with the binding capacity increasing with increase in protein at a given polyphosphate concentration. The importance of the chain length of the polyphosphate was emphasized. If the interaction is at one end of the tripolyphosphate, a pyrophosphate "tail" remains which may be able to form moderately stable calcium complexes. With pyrophosphate, one interaction with a protein site might well eliminate almost all of its complexing power for calcium. It was concluded that for the long chain polyphosphate species there are probably multiple interactions with proteins. The

over-all effect in terms of calcium complex formation will depend on the fraction of sites not bound to the protein and on the arrangement of these "free" sites. deMan (1966) noted that pyrophosphate acts more strongly on protein systems than polyphosphates with longer chain lengths. This was evidenced by greater changes of color and viscosity of skimmilk. Vujicic et al. (1967) indicated that added polyphosphates are quantitatively bound to milk proteins. Inklaar (1967) pointed out in his study on the interaction between polyphosphates and meat that phosphates do not complex with the calcium bound to meat proteins. His results indicate that about 60% of the calcium and 20% of the magnesium naturally present in meat are firmly bound to the meat proteins and are not available to react with added phosphates.

EXPERIMENTAL SECTION

A. Materials utilized:

Beef round steak was purchased daily as required from local supermarkets and stored in a refrigerator 4 - 5 °C until analyzed. Storage time never exceeded 24 hours.

B. Preparation of samples:

1. Preparation of untreated-meat samples:

Fat was trimmed from the meat as much as possible. The samples were then cut in cubes of approximately 1/2 inch. The meat was then homogenized for three minutes in a Waring Blendor at full speed.

The homogenized meat samples were analyzed for soluble inorganic phosphates and nitrogen in the soluble phase of meat.

To determine products of hydrolysis, standard solutions of polyphosphate salts were hydrolyzed using the method of Odagiri and Nickerson (1964). Twenty ml of the solution and 20 ml of a 20% solution of trichloroacetic acid (TCA) were refluxed at a pH of less than 2.5 for 4 hours, converting all the phosphates present to the ortho form, as evidenced by paper chromatographic methods of analysis.

For the soluble phosphate determination, the liquid contained in the homogenized meat was collected employing the pressure filter described by Vasic and deMan (1966) (Figs. 1 and 2). Filtration pressure was 45 lbs/sq. in.

2. Preparation of phosphate-treated meat samples:

The following salt additives were used: sodium pyrophosphate, sodium tripolyphosphate, sodium tetraphosphate, and sodium hexametaphosphate. These salts were added after homogenization in the Waring Blendor for 3 minutes, followed by an additional 2 minutes for complete mixing. The treated samples were analyzed after storage for different periods in a refrigerator.

3. Deactivation of enzymes of phosphate-treated meat samples:

The meat samples were homogenized in the Waring Blendor, then the samples were heated in a steam bath to 80°C for five minutes. After cooling to room temperature, polyphosphates were added and the samples homogenized once again for 2 minutes. The samples were then stored in a refrigerator at 4 - 5°C until analyzed.

C. Methods of Analysis

1. Determination of inorganic phosphate:

Inorganic phosphate was determined by the method of Polley (1949), as modified by deMan (1967).

Solutions prepared from samples for the determination of soluble phosphate were diluted to a phosphorus content within the range of the calibration curve, i.e. containing the equivalent of between 0.2 and 7.0 mg potassium orthophosphate/100 ml. The calibration curve obtained with potassium orthophosphate is shown in Figure 3. The concentration of inorganic phosphate was expressed as mg phosphorus/100 g of sample.

2. Determination of moisture:

Total moisture in the foods was determined by the oven drying method No. 23.002 of the Association of Official Agricultural Chemists (1965).

3. Paper chromatographic separation of mono- and polyphosphates:

Separation was accomplished by the method described by Gassner (1958). The phosphate solutions were spotted on Whatman No. 4 chromatographic paper and developed with a solvent consisting of:

80 ml isopropyl alcohol
5 g trichloroacetic acid
0.3 ml conc. ammonia
40 ml water
40 ml ethyleneglycolmonomethylether

The chromatograms were dried at room temperature and the spots made visible by spraying with a reagent consisting of 1 g ammonium molybdate, 85 ml water, 10 ml N hydrochloric acid, and 5 ml 60% perchloric acid.

4. Soluble nitrogen determination

Nitrogen was determined in duplicate samples of raw and heated meat by the Kjeldahl method (A.O.A.C., 1955).

5. The pH determination:

The pH of meat liquids was determined with a Beckman Zeromatic II pH Meter.

Figure 1. Pressure filtration apparatus assembled.

Figure 2. Pressure filtration apparatus disassembled.

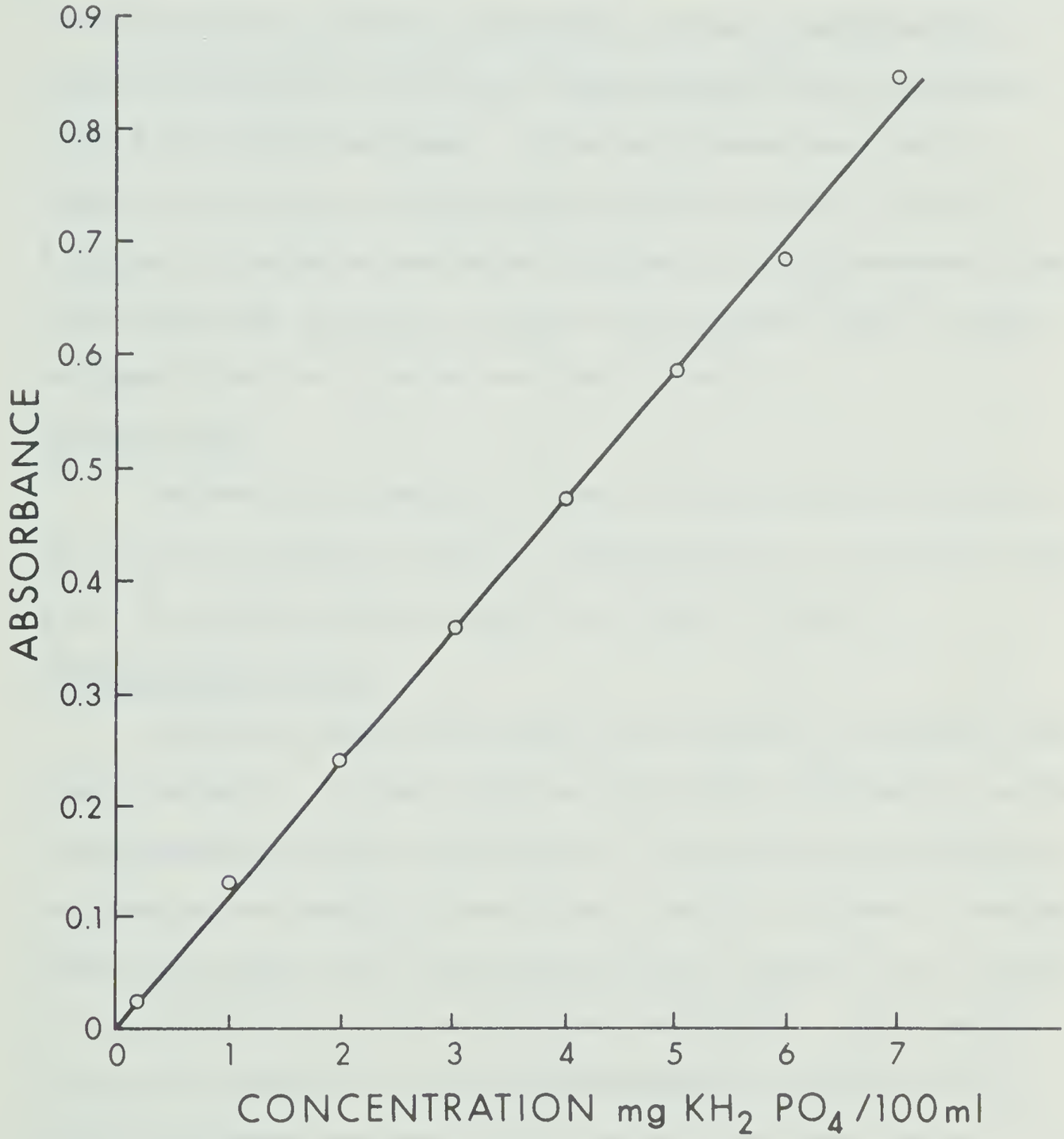


Fig. 3. Standard curve for the determination of inorganic phosphorus in meat.

RESULTS

Phosphates and polyphosphates in solution were separated by ascending paper chromatography. The R_f values of monosodium phosphate, disodium phosphate, sodium pyrophosphate, sodium tripolyphosphate, sodium tetraphosphate, and sodium hexametaphosphate were determined using 0.5% standard soltuions. The values obtained are given in Table 2, and the type of chromatogram obtained is shown in Figure 4. A typical chromatogram showing the hydrolysis of polyphosphates to the ortho form using the method of Odagiri and Nickerson (1964) is shown in Figure 5 and the R_f values are given in Table 2.

Untreated meat:

Untreated meat samples of fresh and heated meat were analyzed for pH, total moisture, soluble P_i (Orthophosphate), and soluble nitrogen. The results of these analyses are listed in Table 3.

Phosphate-treated meat:

Fresh and heated meat samples were subjected to different phosphate treatments. The salts employed were sodium pyrophosphate, sodium tripolyphosphate, sodium tetraphosphate, and sodium hexametaphosphate, each used at two levels, 100 and 200 mg P per 100 g of meat. Fresh and heated meat samples were then analyzed for pH, soluble Pi, and soluble N at intervals of 0, 4, 24 and 48 hours respectively. These results are given in Tables 4-19. Paper chromatographic separation of the soluble phosphates was done with the 200 mg P/100 g meat levels,

at intervals covering periods up to 48 hours from the addition of the polyphosphate salts. These results are given in Figures 8, 11, 14 and 17.

The pH of meat liquids increased in the samples which were treated with sodium pyrophosphate at both levels, as indicated in Tables 4, 5, 6 and 7. This was noted in both fresh and heated meat samples. The addition of pyrophosphate salt to fresh meat also resulted in an increase in the soluble inorganic phosphate content and this continued to increase with time. Heated meat, however, showed a different pattern. At both the low and the high levels there was an immediate increase in soluble inorganic phosphate after addition of pyrophosphate. This increase was in the order of 20 - 30% of the added salt at both levels. No further hydrolysis was the noted in the case of the high level, the inorganic phosphate content remained constant with time. In the case of the low level, however, the increase continued slowly to 30% after four hours, but then remained constant with time. These results are shown in Figures 6 and 7 as in Tables 4, 5, 6 and 7. These tables show as well, a decrease in soluble nitrogen upon addition of pyrophosphate salts to fresh meat. However, an increase was observed in the case of addition of pyrophosphate salts to heated meat. This was followed by an increase in soluble nitrogen in both fresh and heated meat, and there was a continued increase to the end of the experiment.

The paper chromatographic separation, Figure 8, demonstrates the hydrolysis of the pyrophosphate to the ortho-form. This hydrolysis also occurred in the case of the heated meat.

The addition of 100 mg P as sodium tripolyphosphate to fresh and heated meat did not affect the pH. However, there was an increase in the soluble inorganic phosphorus with both treatments immediately after the addition of the tripolyphosphate, as shown in Tables 8 and 9 and Figure 9. This increase continued steadily in the case of fresh meat to the end of the experiment, while the level remained unchanged in the heat treated meat. The soluble nitrogen content showed a different pattern. The soluble nitrogen content of fresh meat increased during the first four hours and then decreased. In the case of heated meat, the soluble nitrogen increased after addition of tripolyphosphate salts, showed a decline at four hours, and then began to increase again.

The addition of 200 mg P as sodium tripolyphosphate, resulted in an increase in the pH of both fresh and heated meat, as shown in Tables 10 and 11. These tables also show a continuing increase in the soluble inorganic phosphorus content of fresh meat, whereas with the treated meat there was an immediate increase after the salt addition, followed by a constant level, as shown in Figure 10.

The soluble nitrogen content decreased in the case of the fresh meat to below the level of untreated fresh meat. There was an increase in the case of the heated meat, followed by a decrease after 24 hours, increasing again after 48 hours. The chromatographic separation of

soluble inorganic phosphates in the sample treated with 200 mg P after 48 hours was in agreement with the quantitative analysis as shown in Figure 11.

No change in pH was observed in fresh and heated meat samples treated with 100 mg P as sodium tetraphosphate as shown in Tables 12 and 13. These tables also show an immediate increase in the soluble nitrogen content after treatment with tetraphosphates and this was followed by a decrease until the end of the experiment. The soluble inorganic phosphate continued to increase with the fresh meat. There was an immediate increase in the soluble inorganic phosphorus with the heated meat and the level remained unchanged until the end of the experiment, as shown in Figure 12.

Tables 14 and 15 and Figure 13 give the results of the addition of 200 mg P as sodium tetraphosphate. The pH of fresh meat increased after the addition of salt, and this was followed by a decrease to the level of untreated meat. The heated meat pH increased after the salt addition and this was followed by a decrease after 4 hours, the pH then remained constant. The soluble nitrogen content increased with both fresh and heated meats. The soluble inorganic phosphorus continued to increase in the case of fresh meat, whereas with heated meat there was an immediate increase followed by constant levels indicating no hydrolysis occurred after the initial rise in orthophosphate content. The chromatogram obtained at 48 hours after the salt addition is shown in Figure 14, indicating more complete hydrolysis in the fresh than in the heated meat.

The results obtained after adding 100 mg P as sodium hexametaphosphate to fresh and heated meats, are given in Tables 10 and 17 and Figure 15. The pH of the fresh meat decreased to a lower level than in the untreated meat, whereas the pH of the heated meat remained the same. There was an increase in the level of soluble nitrogen in both fresh and heated meat. The fresh meat showed an initial lowering followed by an increase in soluble nitrogen. The soluble inorganic phosphorus content of fresh meat steadily increased while in the heated meat there was an increase up to 24 hours followed by a constant level until the end of the experiment.

The results of adding 200 mg P as sodium hexametaphosphate to fresh and heated meat are given in Tables 18, 19 and Figure 16. The pH remained unchanged in both cases. There was an increase in the level of soluble inorganic phosphorus in fresh meat to 48 hours, no change was observed in the case of heated meat after 0 hours. The soluble nitrogen content increased in both cases. The chromatogram obtained after 48 hours is shown in Figure 17, demonstrating the hydrolysis of sodium hexametaphosphate in the case of fresh meat, and the lack of hydrolysis in the case of heated meat.

Table 2. R_f values of ortho- and polyphosphates.

Salt	R_f	
	Before hydrolysis	After hydrolysis
Monosodium phosphate	0.85	
Disodium phosphate	0.82	
Sodium pyrophosphate	0.58	0.80
Sodium tripolyphosphate	0.38	0.82
Sodium tetraphosphate	0.00	0.85
Sodium hexametaphosphate	0.00	0.78

Fig. 4. Paper chromatogram of (A) monosodium phosphate solution, (B) disodium phosphate solution, (C) sodium pyrophosphate solution, (D) sodium tripolyphosphate solution, (E) sodium tetraphosphate solution, and (F) sodium hexametaphosphate solution.

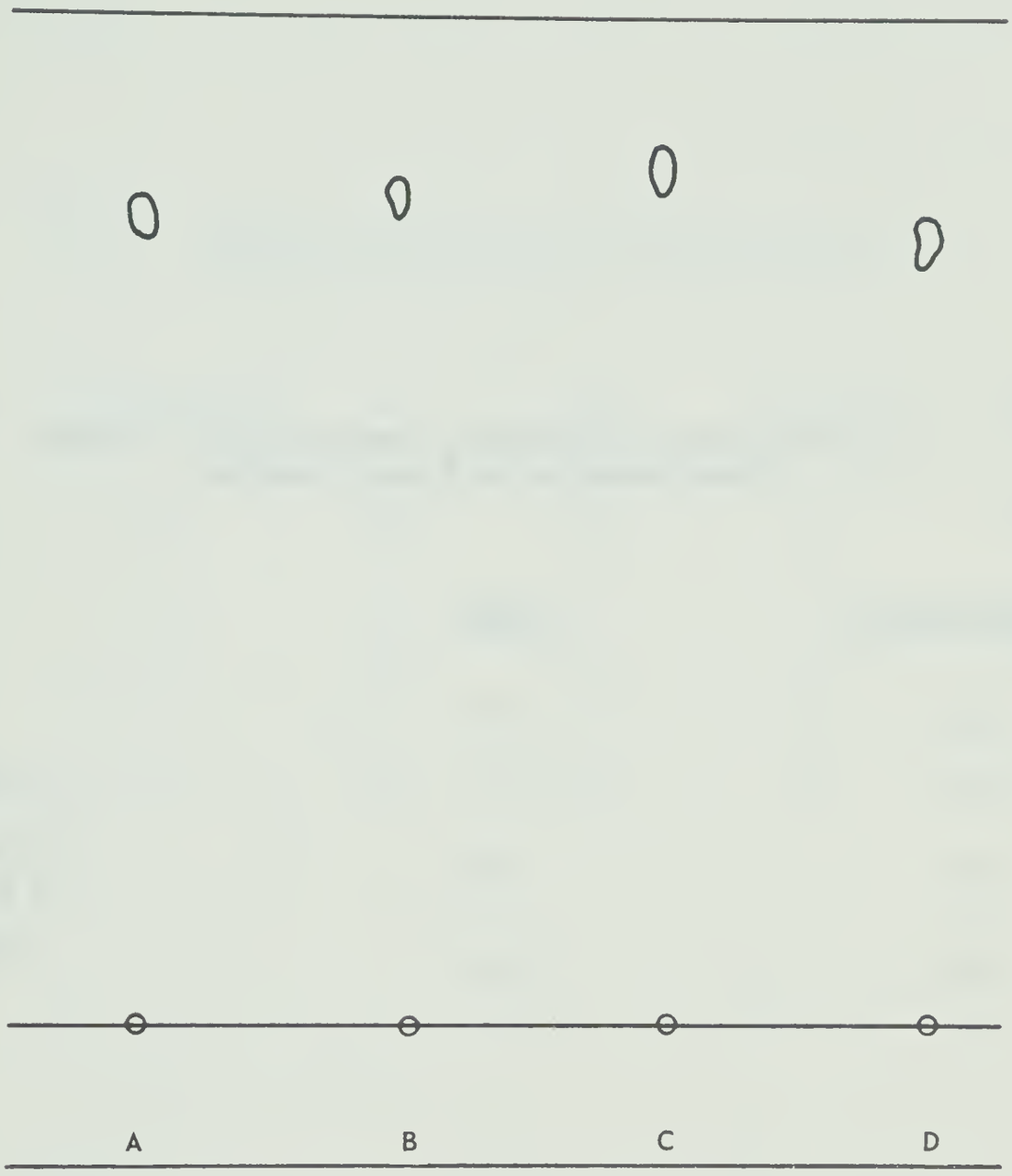


Fig. 5. Paper chromatogram of (A) sodium pyrophosphate solution after hydrolysis, (B) sodium tripolyphosphate solution after hydrolysis, (C) sodium tetraphosphate solution after hydrolysis, and (D) sodium hexametaphosphate solution after hydrolysis.

Table 3. pH, moisture content, and concentration of soluble P_i and N in untreated meat.

	Fresh	After Heating
pH	5.8	6.01
Moisture, %	75	72.6
Soluble P_i mg/100 g	116	112
Soluble N,%	0.66	0.59

Table 4. Effect of addition of 100 mg P as sodium pyrophosphate to 100 g of fresh meat.

Time hrs	Storage Temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.00	126	10	0.53
4	4-5	6.00	143	27	0.63
24	4-5	6.00	198	82	0.88
48	4-5	6.00	201	85	0.75

Table 5. Effect of addition of 100 mg P sodium pyrophosphate to 100 g of meat, heated to 80°C

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N
0	4-5	6.2	135	23	0.63
4	4-5	6.2	142	30	0.63
24	4-5	6.2	142	30	0.68
48	4-5	6.2	142	30	0.69

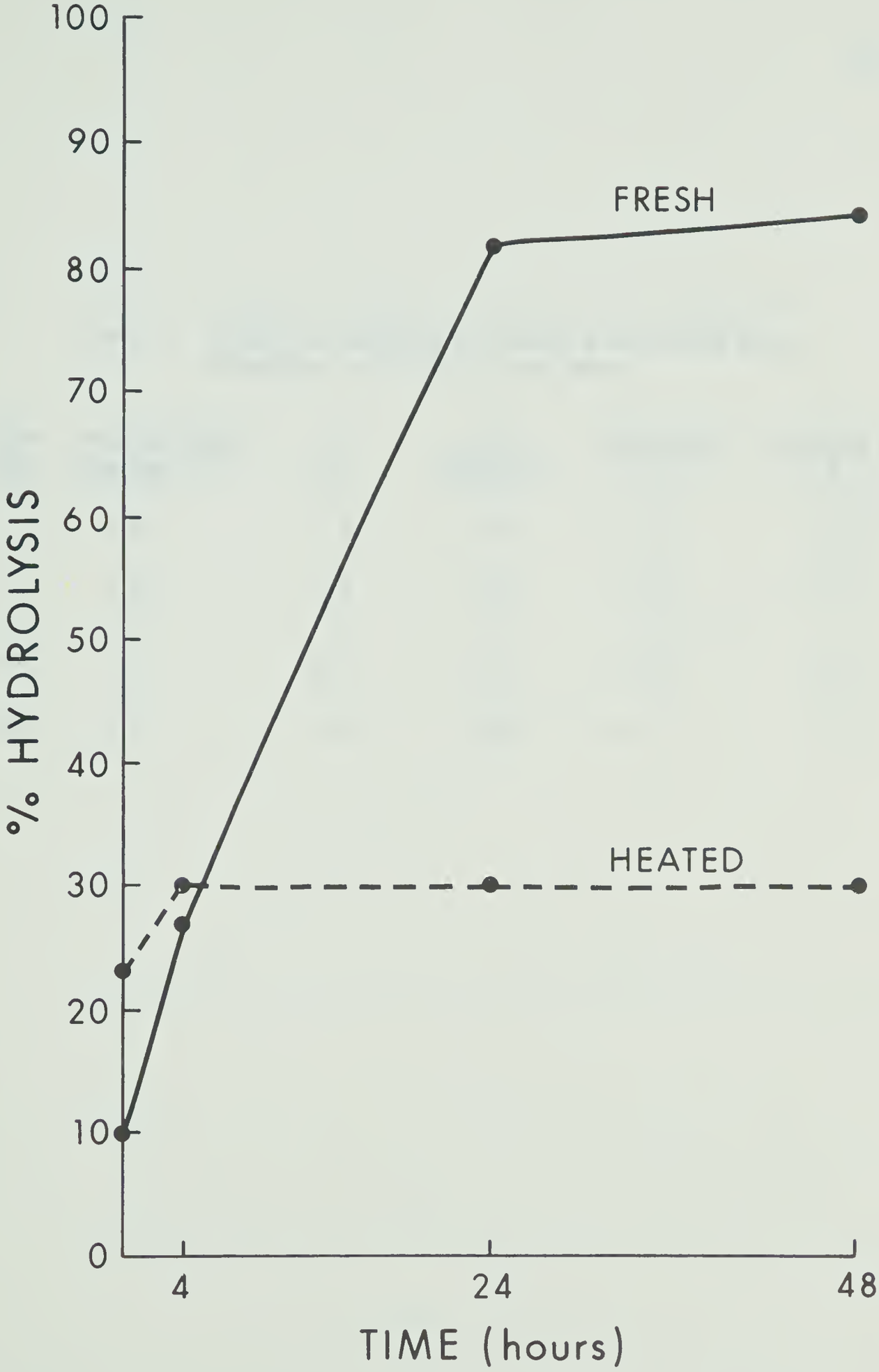


Fig. 6. Effect of addition of 100 mg P as sodium pyrophosphate to 100 g meat.

Table 6. Effect of addition of 200 mg P as sodium pyrophosphate to 100 g of fresh meat.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.3	157	20.5	0.63
4	4-5	6.3	157	20.5	0.62
24	4-5	6.3	197	40.5	0.62
48	4-5	6.3	232	58	0.65

Table 7. Effect of addition of 200 mg P as sodium pyrophosphate to 100 g of meat heated to 80°C.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.4	152	20	0.68
4	4-5	6.4	152	20	0.69
24	4-5	6.4	152	20	0.65
48	4-5	6.4	152	20	0.69

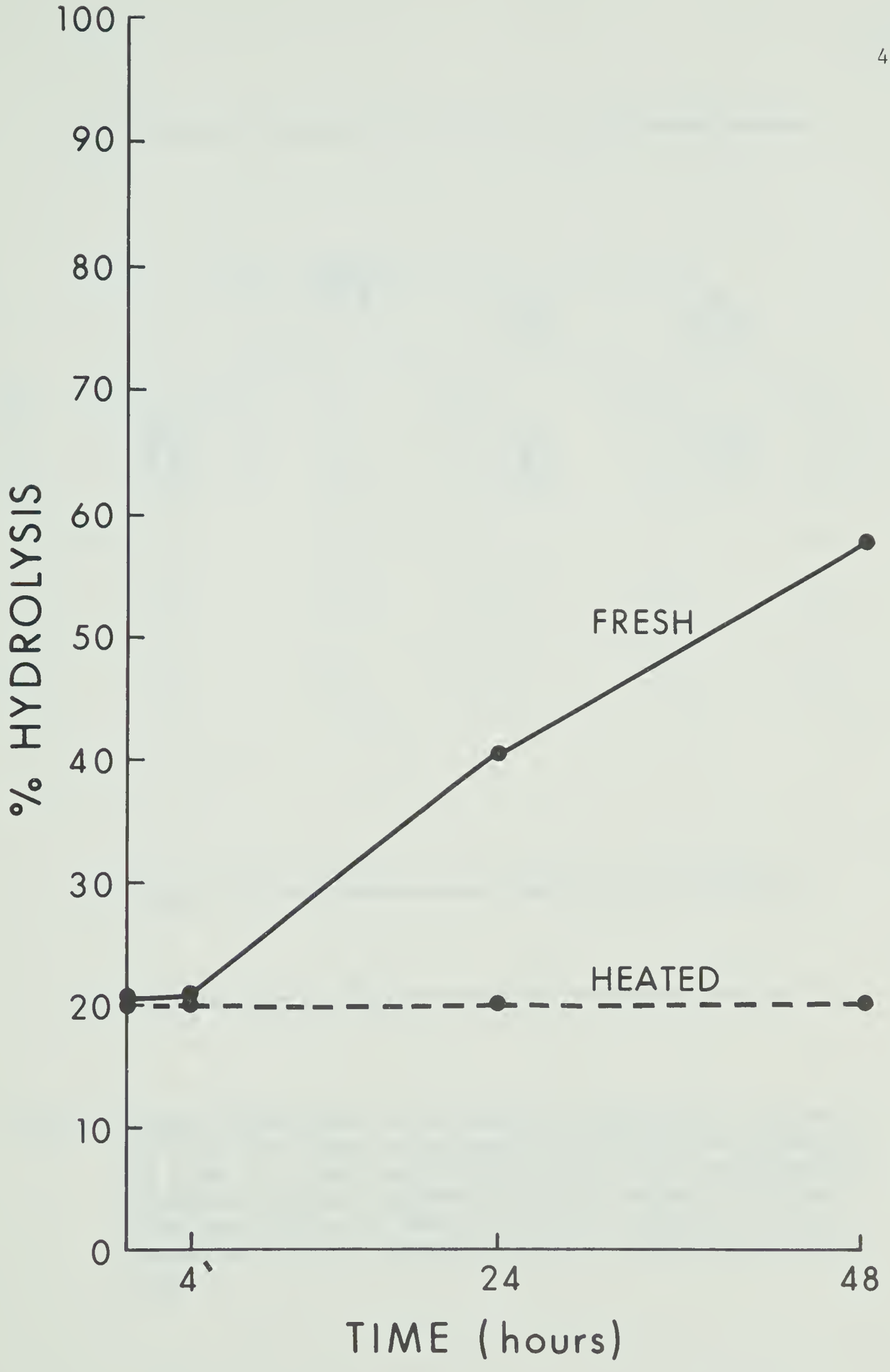
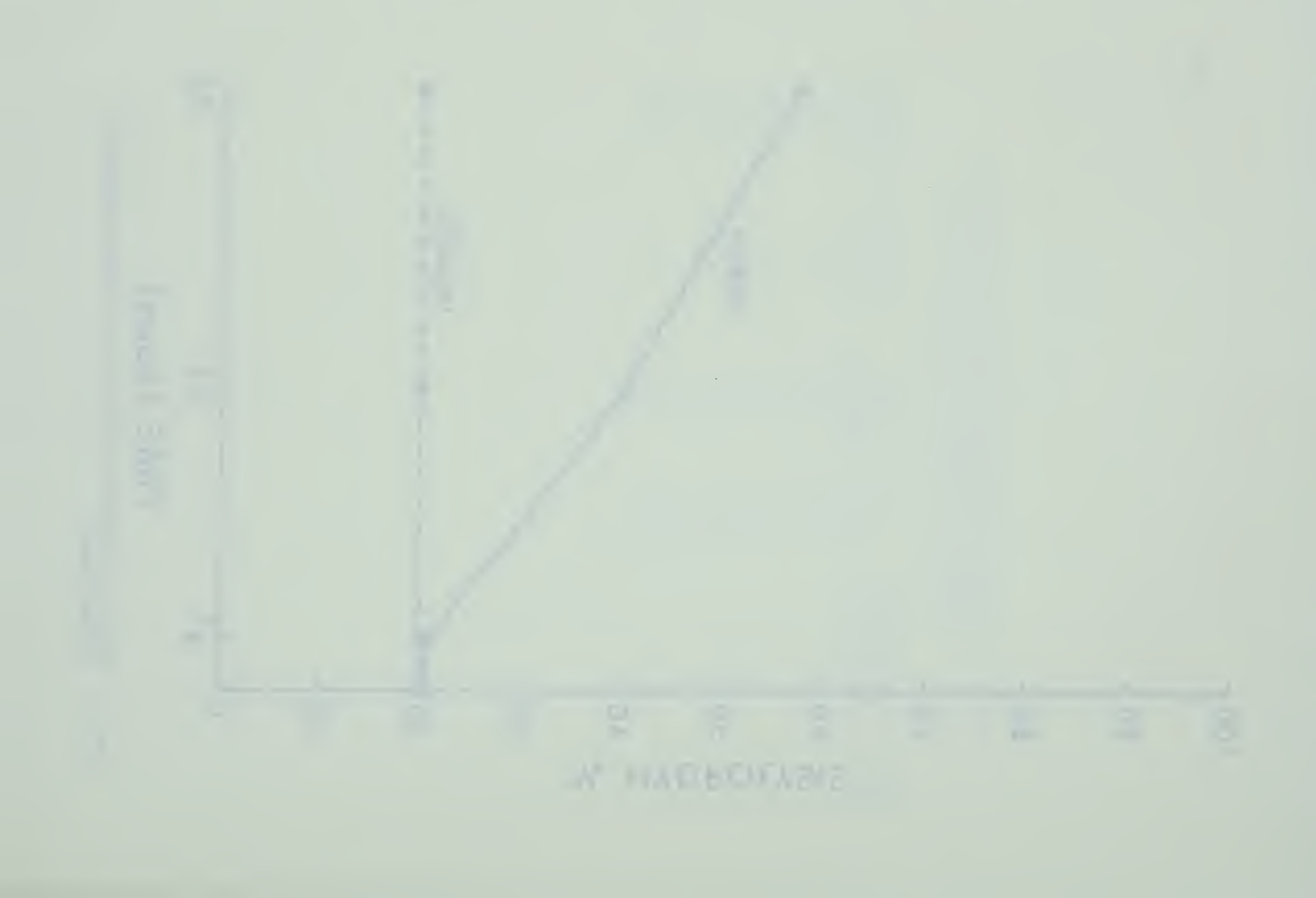


Fig. 7. Effect of addition of 200 mg P as sodium pyrophosphate to 100 g meat.



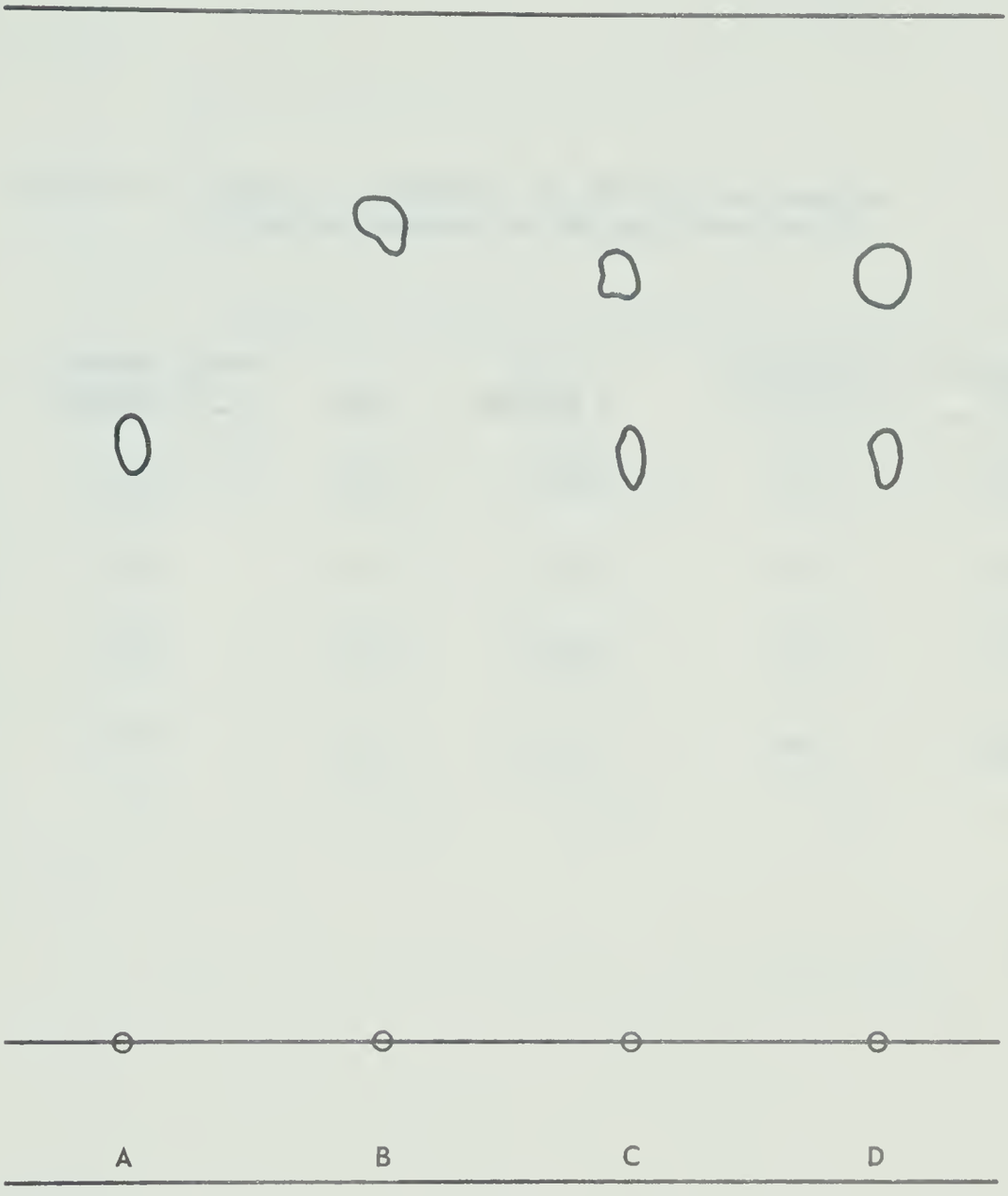


Fig. 8. Paper chromatogram of (A) sodium pyrophosphate solution R_f 0.58, (B) sodium pyrophosphate solution after hydrolysis R_f 0.80, (C) fresh meat treated with sodium pyrophosphate separated into two components with R_f values of 0.74 and 0.56, and (D) heated meat treated with the same salt and separated into two components with R_f values of 0.74 and 0.56.

Table 8. Effect of addition of 100 mg P as sodium tripolyphosphate to 100 gm fresh meat.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	5.8	163	47	0.73
4	4-5	5.8	204	88	0.76
24	4-5	5.8	205	89	0.73
48	4-5	5.8	222	96	0.69

Table 9. Effect of addition of 100 mg P as sodium tripolyphosphate to 100 g of meat heated to 80°C.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.0	145	33	0.66
4	4-5	6.0	145	33	0.58
24	4-5	6.0	145	33	0.66
48	4-5	6.0	145	33	0.69

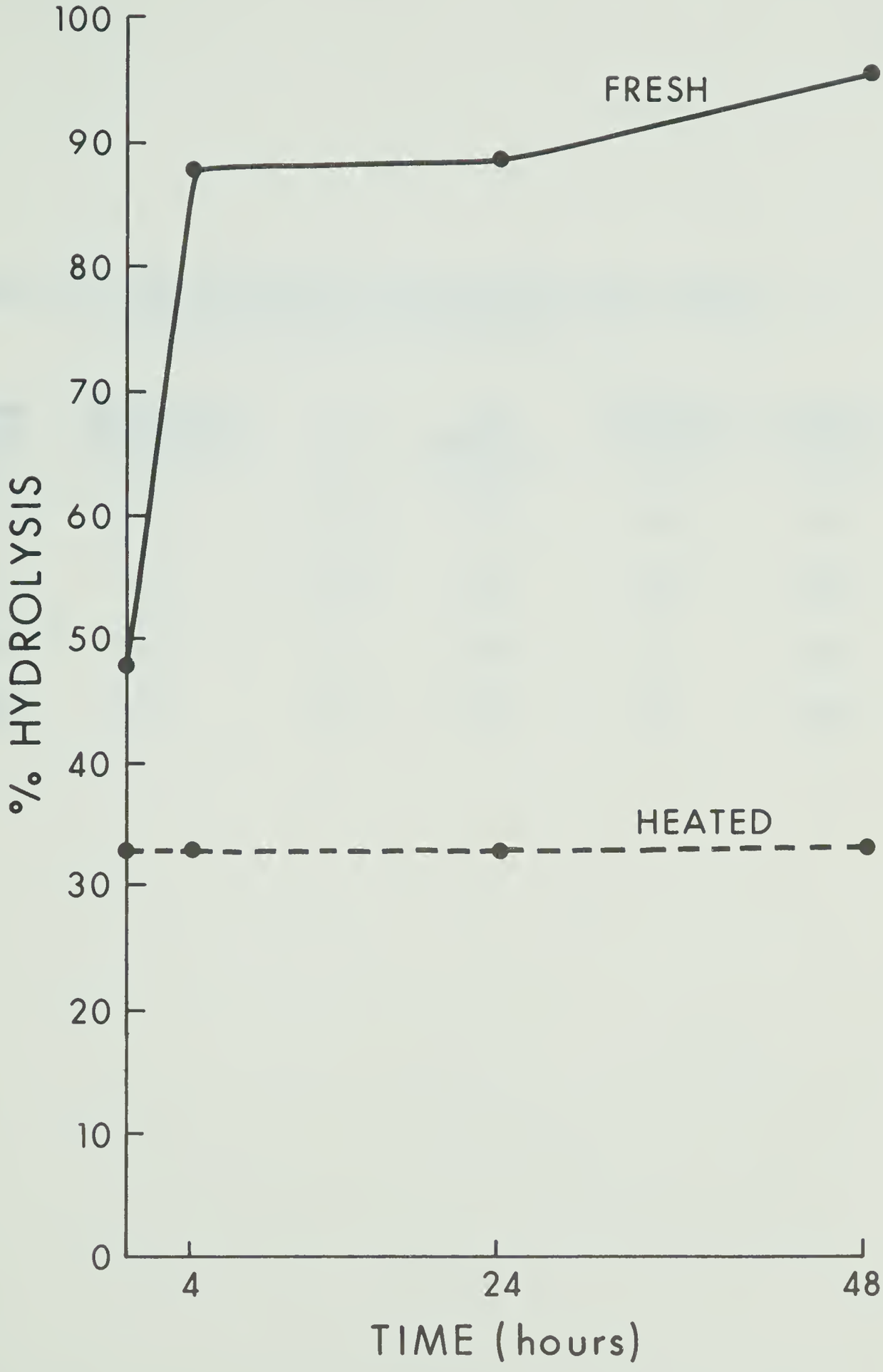


Fig. 9. Effect of addition of 100 mg P as sodium tripolyphosphate to 100 g meat.

Table 10. Effect of addition of 200 mg P as sodium tripolyphosphate to 100 g of fresh meat.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.00	197	40.5	0.53
4	4-5	6.00	225	49.5	0.52
24	4-5	6.1	238	61	0.52
48	4-5	6.1	272	78	0.54

Table 11. Effect of addition of 200 mg P as sodium tripolyphosphate to 100 g of meat heated to 80°C.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.2	138	13	0.62
4	4-5	6.2	138	13	0.63
24	4-5	6.2	138	13	0.58
48	4-5	6.2	138	13	0.63

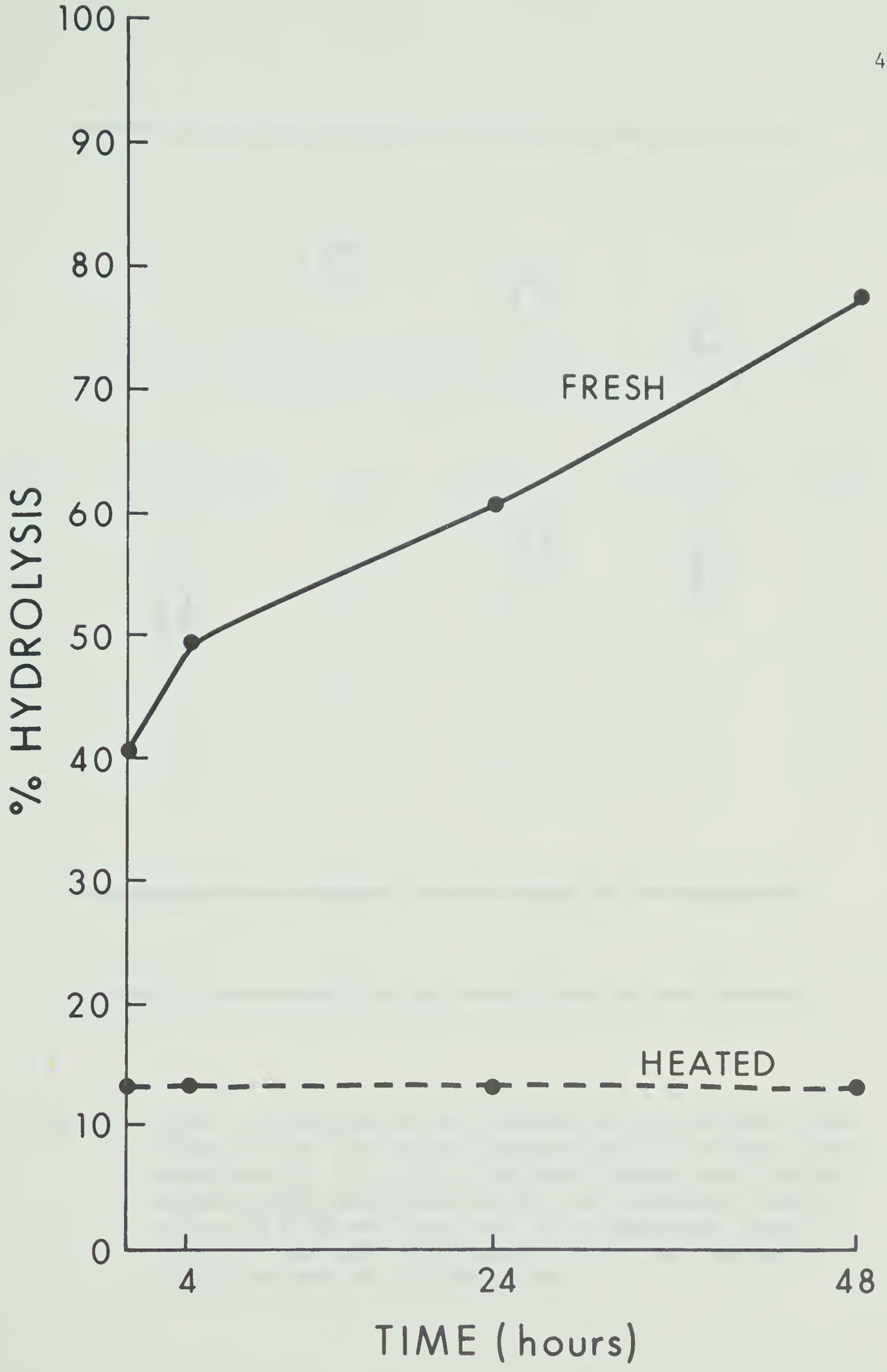
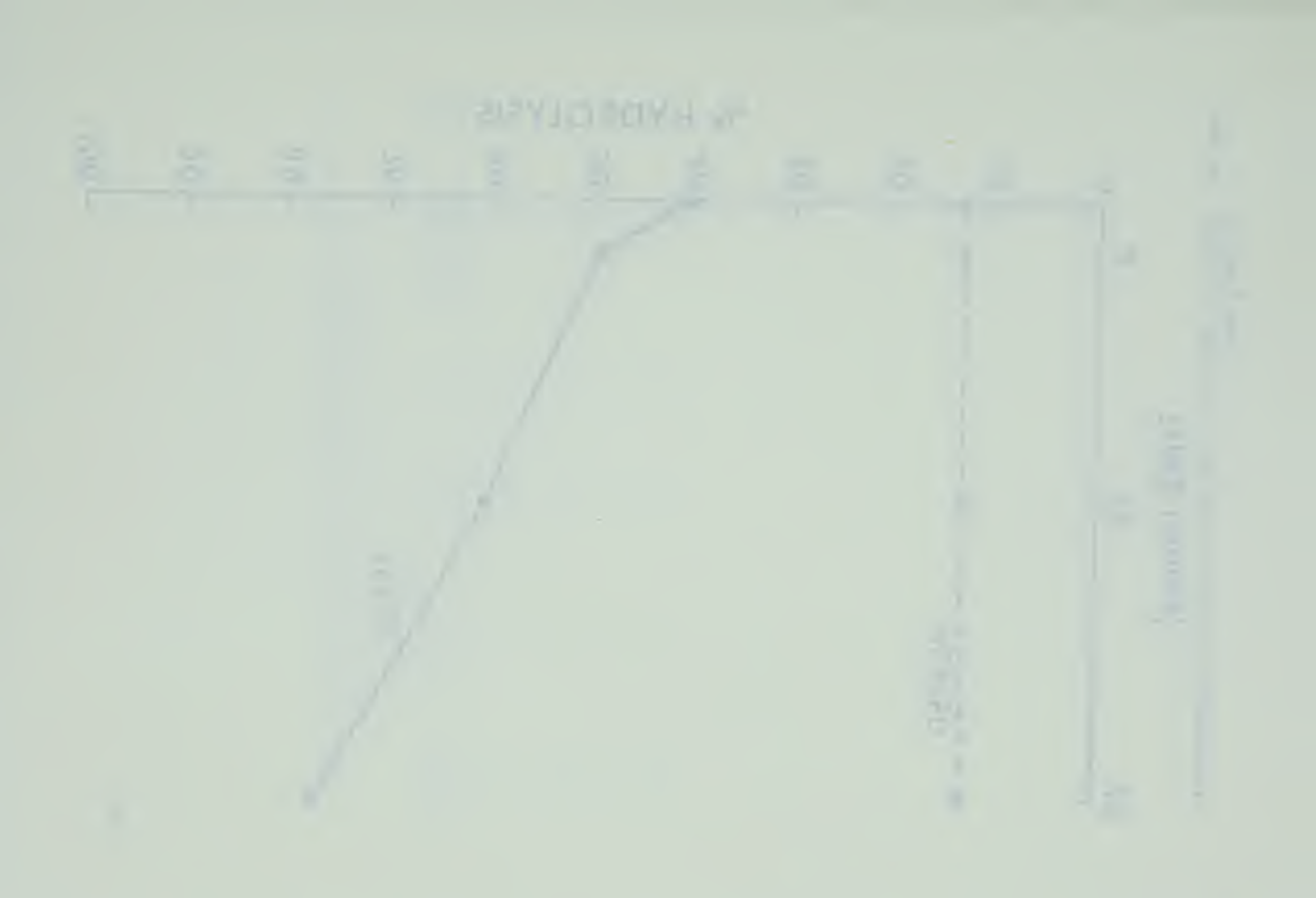


Fig. 10. Effect of addition of 200 mg P as sodium tripolyphosphate to 100 g meat.



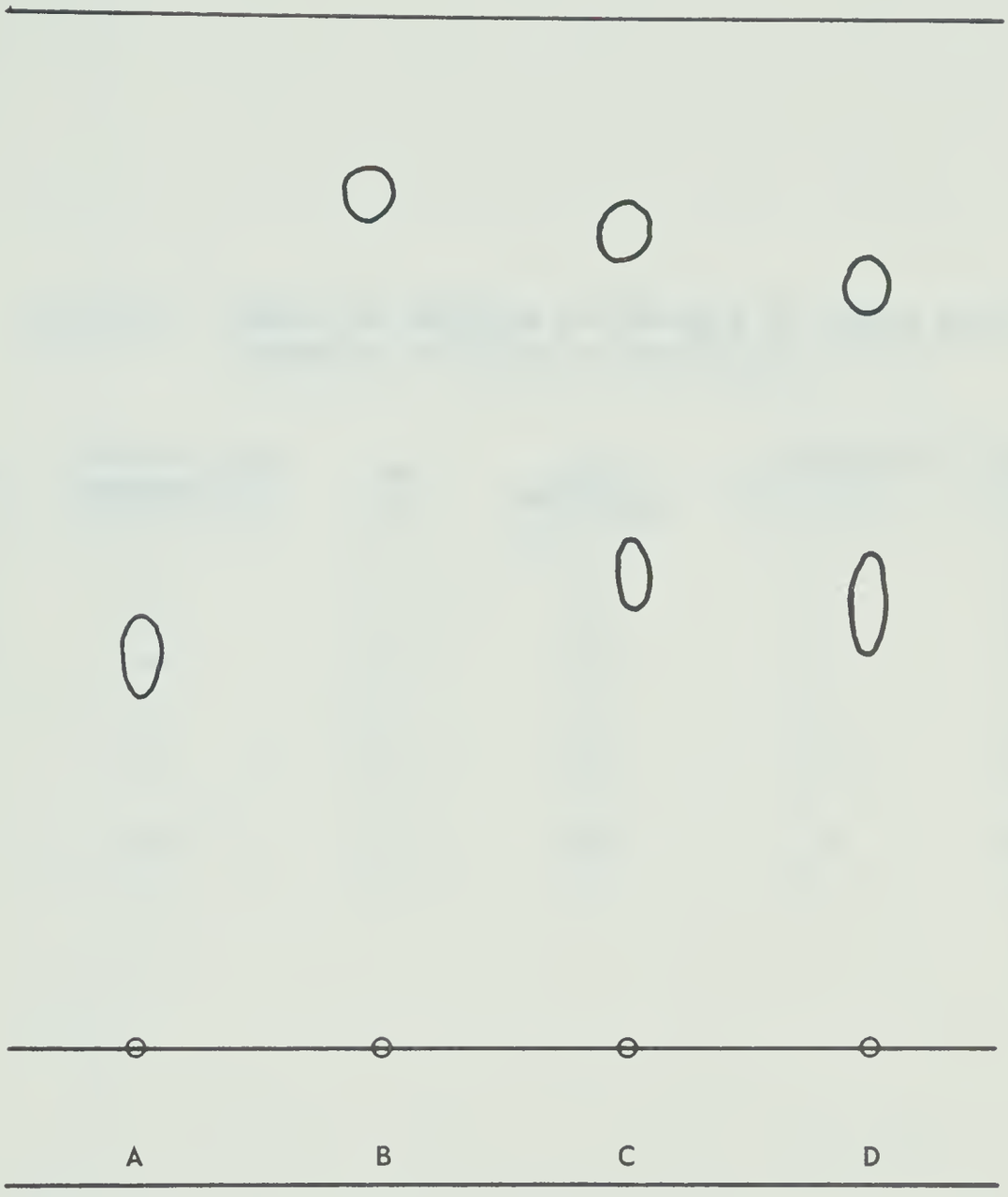


Fig. 11. Paper chromatogram of (A) sodium tripolyphosphate solution R_f 0.38, (B) sodium tripolyphosphate solution after hydrolysis R_f 0.82, (C) fresh meat treated with sodium tripolyphosphate separated into two components with R_f values of 0.80 and 0.46, and (D) heated meat treated with the same salt and separated into two components with R_f values of 0.72 and 0.44.

Table 12. Effect of addition of 100 mg P as sodium tetraphosphate to 100 gm of fresh meat.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	5.8	143	27	0.74
4	4-5	5.8	157	41	0.70
24	4-5	5.8	177	61	0.54
48	4-5	5.8	184	68	0.61

Table 13. Effect of addition of 100 mg P as sodium tetraphosphate to 100 g of meat heated at 80°C.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.00	132	20	0.65
4	4-5	6.00	132	20	0.64
24	4-5	6.00	132	20	0.63
48	4-5	6.00	132	20	0.61

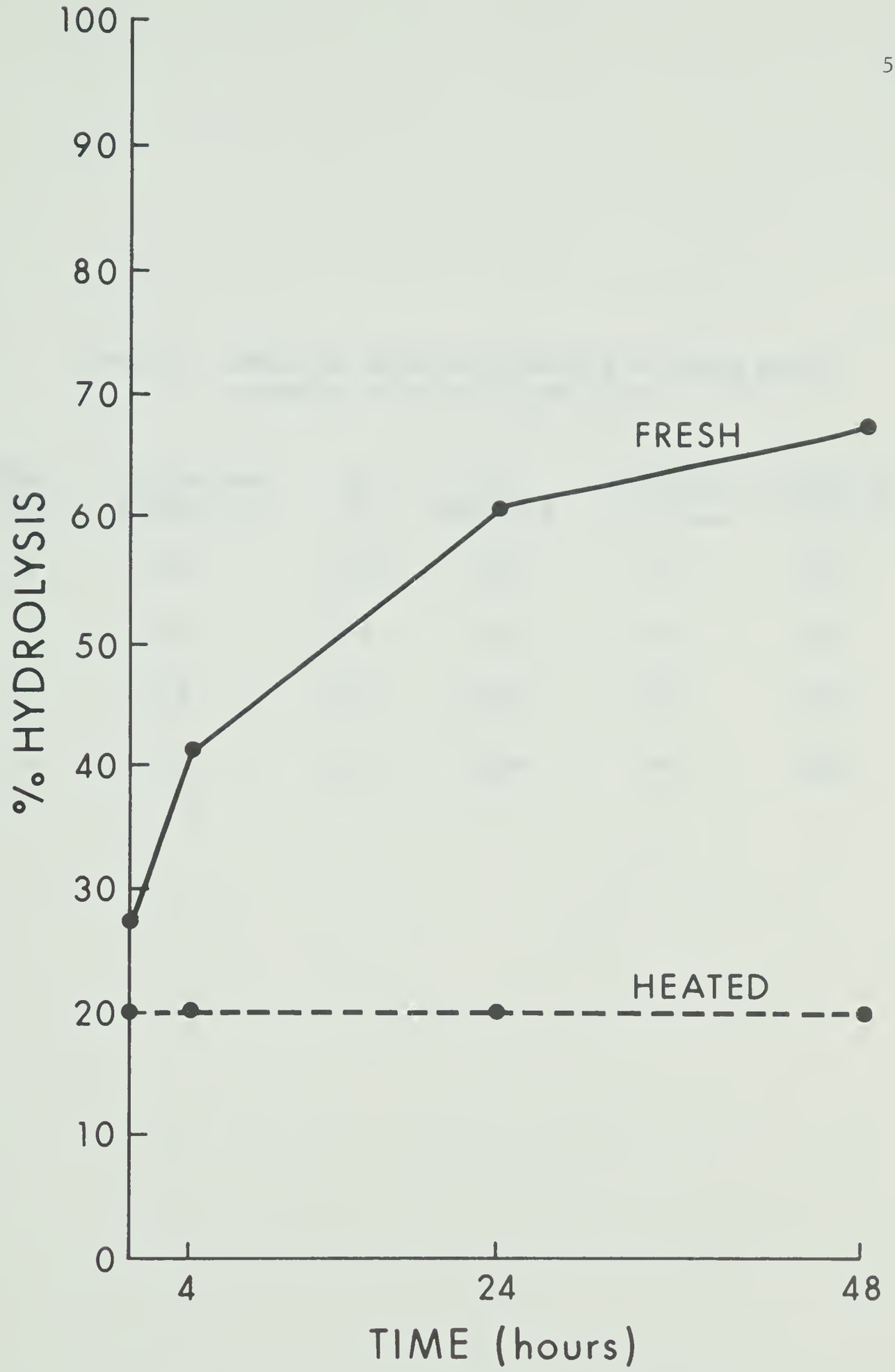


Fig. 12. Effect of addition of 100 mg P as sodium tetraphosphate to 100 g meat.

Table 14. Effect of addition of 200 mg P as sodium tetraphosphate to 100 g of fresh meat.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.00	184	34	0.63
4	4-5	5.8	198	41	0.62
24	4-5	5.8	252	68	0.59
48	4-5	5.8	286	85	0.68

Table 15. Effect of addition of 200 mg P as sodium tetraphosphate to 100 g of meat heated to 80°C.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.3	132	10	0.60
4	4-5	6.1	132	10	0.62
24	4-5	6.1	132	10	0.62
48	4-5	6.1	132	10	0.65

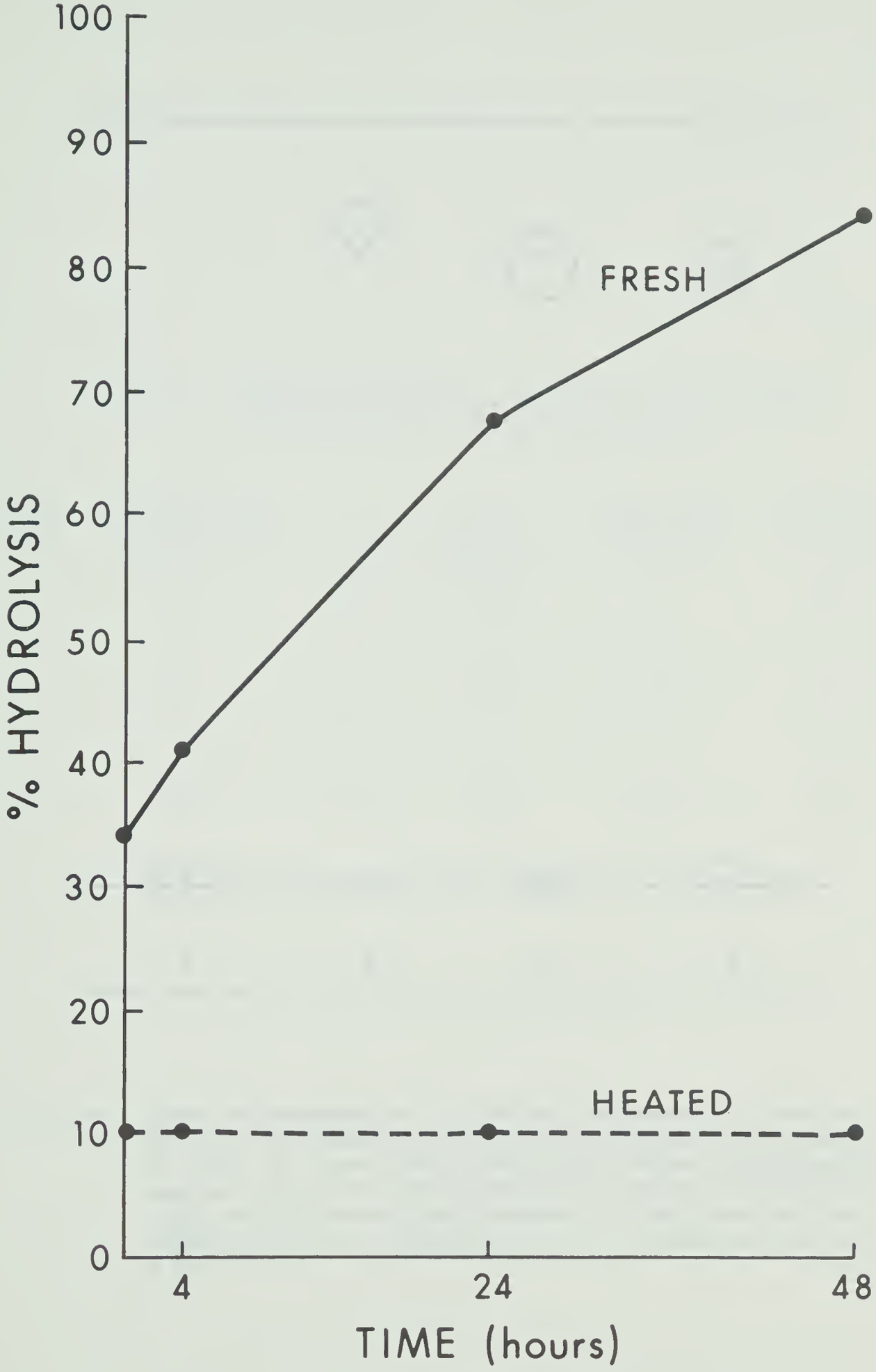


Fig. 13. Effect of addition of 200 mg P as sodium tetraphosphate to 100 g meat.

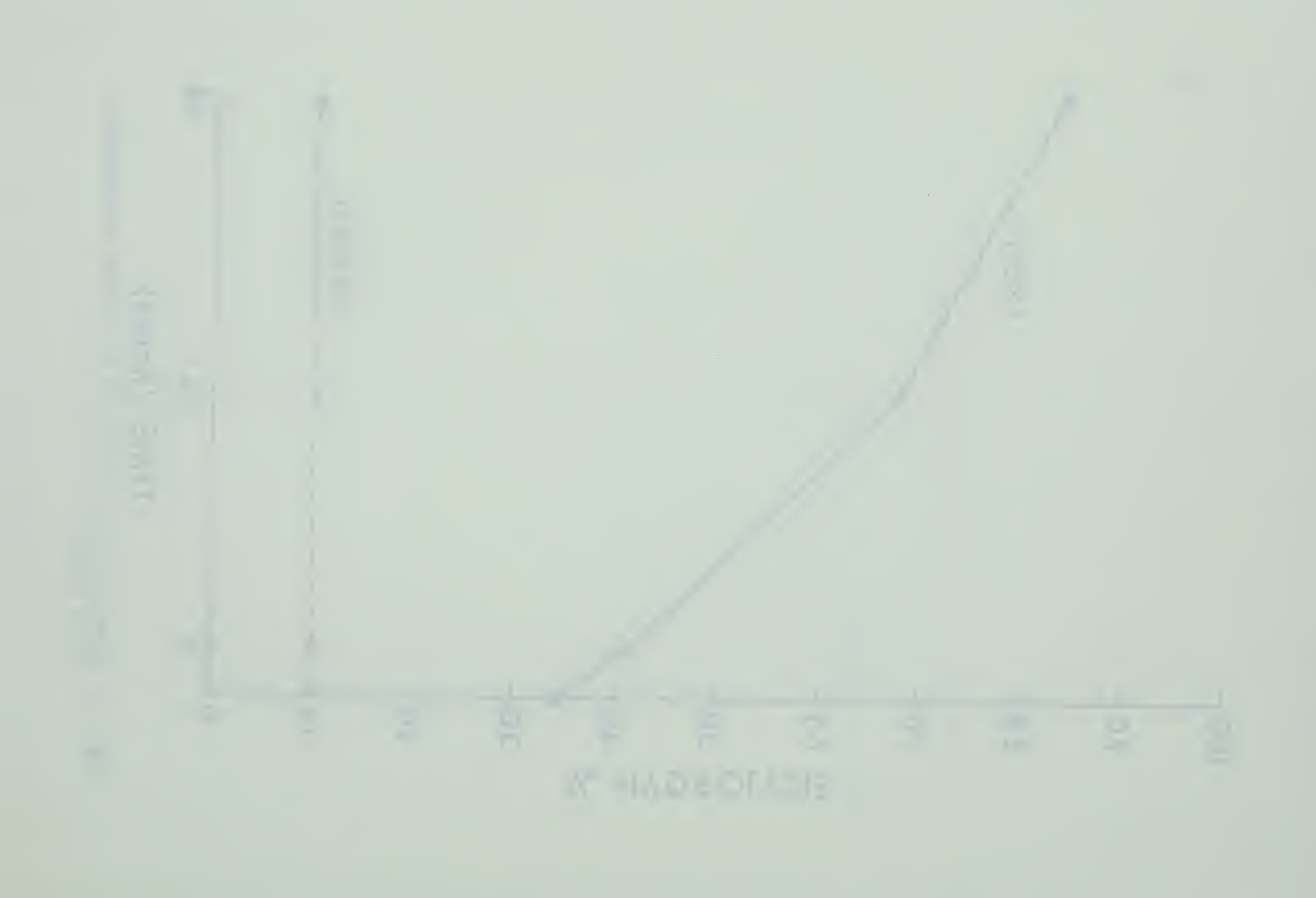


Fig. 14. Paper chromatogram of (A) sodium tetraphosphate solution R_f 0.00, (B) sodium tetraphosphate solution after hydrolysis R_f 0.85, (C) fresh meat treated with sodium tetraphosphate separated into two components with R_f values of 0.80 and 0.00, and (D) heated meat treated with the same salt and separated into two components with R_f values of 0.80 and 0.00.

Table 16. Effect of addition of 100 mg P as sodium hexametaphosphate to 100 g of fresh meat.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	5.7	150	34	0.65
4	4-5	5.7	157	41	0.72
24	4-5	5.7	170	54	0.72
48	4-5	5.7	177	61	0.72

Table 17. Effect of addition of 100 mg P as sodium hexametaphosphate to 100 g of meat heated to 80°C.

Time hrs	Storage temperature °C	pH	Pi mg/100 G	Hydrolysis %	Soluble N %
0	4-5	6.00	119	7	0.59
4	4-5	6.00	125	13	0.63
24	4-5	6.00	145	33	0.64
48	4-5	6.00	145	33	0.65

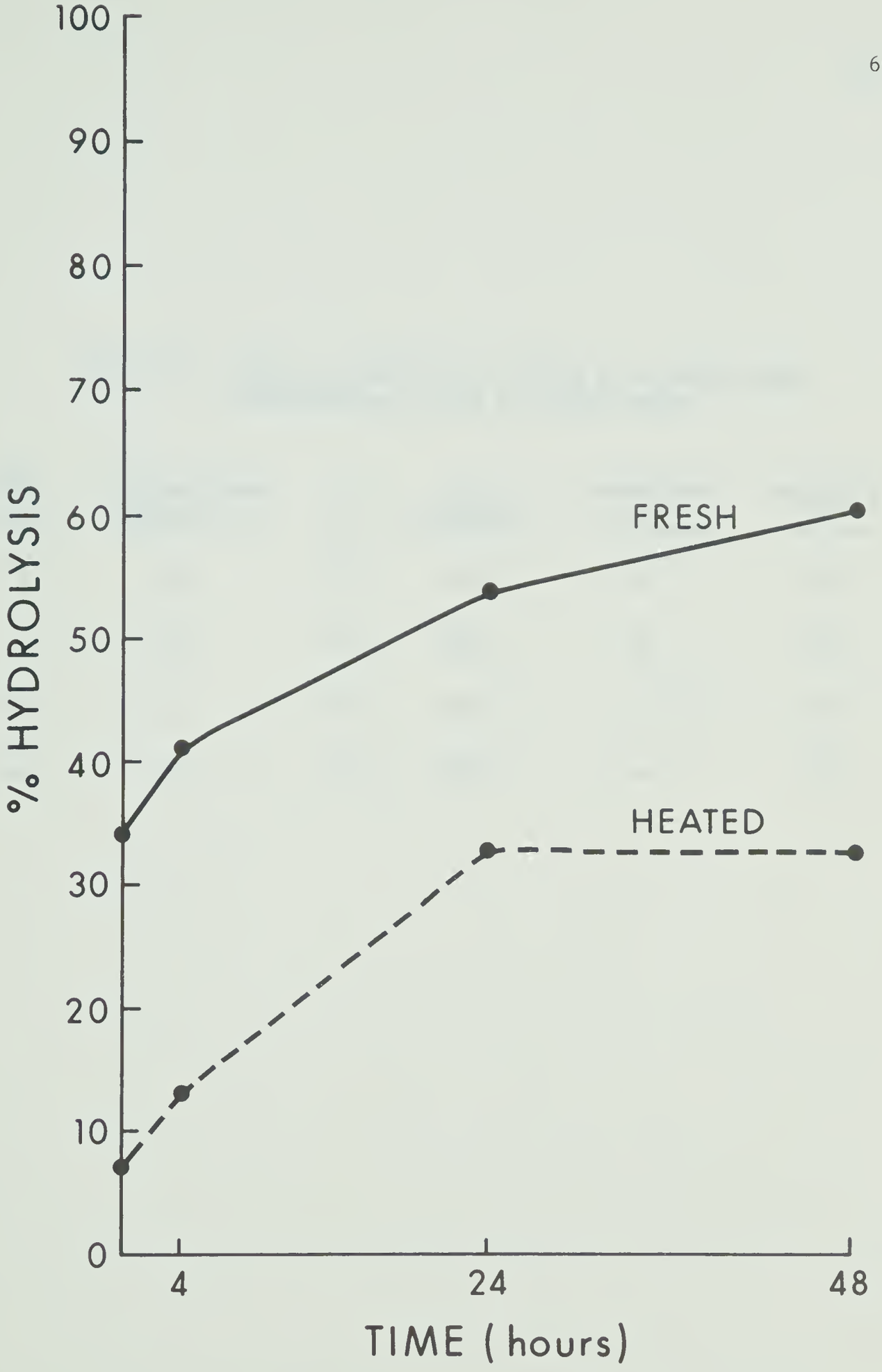


Fig. 15. Effect of addition of 100 mg P as sodium hexametaphosphate to 100 g of meat.

Table 18. Effect of addition of 200 mg P as sodium hexametaphosphate to 100 g of fresh meat.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	5.8	170	27	0.54
4	4-5	5.8	184	34	0.63
24	4-5	5.8	198	41	0.65
48	4-5	5.8	208	46	0.76

Table 19. Effect of addition of 200 mg P as sodium hexametaphosphate to 100 g of meat heated to 80°C.

Time hrs	Storage temperature °C	pH	Pi mg/100 g	Hydrolysis %	Soluble N %
0	4-5	6.00	145	16.5	0.62
4	4-5	6.00	145	16.5	0.66
24	4-5	6.00	145	16.5	0.64
48	4-5	6.00	145	16.5	0.63

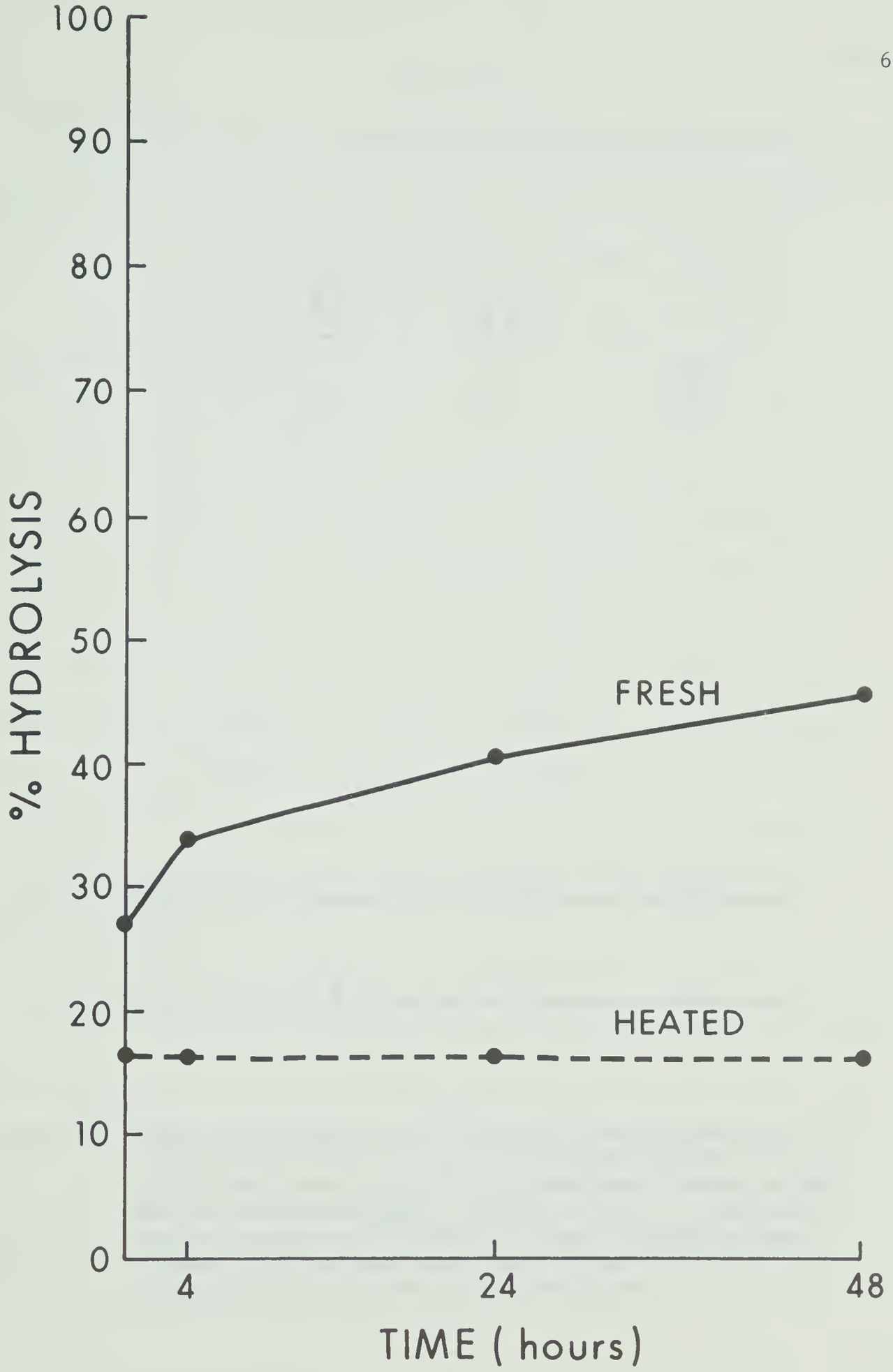


Fig. 16. Effect of addition of 200 mg P as sodium hexametaphosphate to 100 g of meat.

Fig. 17. Paper chromatogram of (A) sodium hexametaphosphate solution R_f 0.00, (B) sodium hexametaphosphate solution after hydrolysis R_f 0.78, (C) fresh meat treated with sodium hexametaphosphate separated into two components with R_f values of 0.77 and 0.00, and (D) heated meat treated with the same salt and separated into two components with R_f values of 0.68 and 0.00.

DISCUSSION

This investigation was undertaken to obtain information about the fate of condensed phosphates added to meat, and their effect on the soluble nitrogen content and the pH of the meat samples.

The results obtained from polyphosphate treated meat indicated that an appreciable hydrolysis takes place immediately after the addition of the salts and continues with time. Consequently, there is an increase in soluble inorganic phosphorus (orthophosphate). The extent of hydrolysis found after 48 hours in all types of polyphosphates tested varied from 46 to 96%. The most extensive hydrolysis (96%) was obtained with tripolyphosphate, which contains three PO_4 residues similar to those of ATP. This observation may be explained by the fact that the enzyme systems responsible for ATP hydrolysis find tripolyphosphate a more similar substrate than other polyphosphates used. The lowest percentage of hydrolysis (46%), was obtained in the case of hexametaphosphate which has the most complex structure of all the condensed phosphates used.

The observed results are in agreement with the work of Cleoceri and Lee (1965a), Sawyer (1952), Karl-Kroupa *et al*. (1957), Englebrecht and Morgan (1959), and Scharpf and Kichline (1967), who reported that the presence of micro-organisms in solutions of condensed phosphates caused an increased rate of hydrolysis of these phosphates. Similarly Harold and Harold (1965) showed that at least four enzyme systems have

been isolated from microbial cells that degrade inorganic polyphosphates. These observations can assist in the explanation of the results obtained in this investigation. In the present experiments it was evident that pretreatment of meat samples by heat to deactivate the enzyme systems substantially reduced the extent of hydrolysis of polyphosphates.

The quantitative results obtained were supported by qualitative analysis using paper chromatography, at 4 and 48 hours after addition of polyphosphates. Hydrolysis in all cases resulted in formation of orthophosphate as the sole product, identified by R_f value. Further, it was shown that in the case of heated meat there was some initial hydrolysis. This hydrolysis can not be a result of enzyme activity in the meat as the meat was heated for 5 minutes at 80°C which should deactivate all the enzymes involved in hydrolysis. Other factors such as the complexing cations, the pH and the ionic environment must be responsible for the observed hydrolysis. Additionally the amount of polyphosphates hydrolyzed at both concentration levels was approximately the same and the hydrolysis did not increase with time, indicating that some fixed quantity of polyphosphates were hydrolyzed non enzymatically, independent of the amount of polyphosphates added.

Some small variations in soluble nitrogen have been noticed with time in all heated samples. Also, some changes in soluble nitrogen took place in the case of some fresh meat samples, while it remained almost constant in others. For example, the hexametaphosphate treated

meat showed an increase from 0.54 up to 0.76 in the case of the 200 mg level, and 0.65-0.72 in case of 100 mg treated samples. The pyrophosphate treated samples showed a similar increase in case of the 100 mg level, while the 200 mg sample remained more or less constant. This is in agreement with the work done by Bendall (1954, 1958), Swift and Ellis (1956), Monk et al. (1964), and Love and Abel (1966), who reported that there was a dissolution of proteins when meat was treated with phosphates. Perhaps the added salts affect the solubility of meat proteins in different ways and in the case of heated samples, heat denaturation of protein certainly would be reflected by changes in solubility behavior.

The results of pH measurements appear in tables of data on each specific system. Heat treatment results in a decrease in acidity of meats. For the polyphosphates added, generally a decrease in acidity is notable in relation to the amount of hydrolysis. This is probably the effect of the buffering capacity of orthophosphate, the product of hydrolysis of all polyphosphates used.

The results of these experiments show conclusively that enzyme systems in fresh meat retain sufficient activity to hydrolyze polyphosphate salts added to the meat. Quantitative analysis of orthophosphate show definitely that these enzyme systems are deactivated by heat treatment. This study indicates that the efficiency of added polyphosphates in preserving quality of meat products is increased by heat treatment of meat before addition of these salts.

REFERENCES

A.O.A.C. (1955).
Official Methods of the Association of Official Agricultural Chemists. 8th edition, Washington: Association of Official Agricultural Chemists, p. 805.

A.O.A.C. (1965).
Official Methods of the Association of Official Agricultural Chemists. 10th edition. Washington: Association of Official Agricultural Chemists, p. 327.

Bamann E. and M. Meisenheimer. (1938).
Umwandlung von metaphosphate in orthophosphate under dem einfuss von metall-oxyden und hydroxyden - III: Mitteil uber "phosphatatiache" wirkungen von hydrogelen.
Berichte der Deutschen Chemischen, Gessellschaft, 71B, 2086-2089.

Bell, Russel N. (1947).
Hydrolysis of dehydrated sodium phosphates.
Industrial and Engineering Chemistry 39 (2), 136-140.

Bell, Russell N. (1952).
Preparation of sodium tetrametaphosphate.
Industrial and Engineering Chemistry. 44, 568-572.

Bendall, J.R. (1954).
The swelling effect of polyphosphates on lean meat.
J. Sci. Food Agric., 5, October, 468-475.

Bendall, J.R. (1958).
Theswelling effect of polyphosphates on lean meat.
Chemistry and Industry, 379-380.

Briggs, D.R. (1940).
The metaphosphoric acid - protein reaction.
The Journal of Biological Chemistry, 134, 261-272.

Campbell, D.O. and M.L. Kilpatrick. (1954).
A kinetic study of the hydrolysis of pyrophosphates.
Journal of the American Chemical Society, 76, 893-901.

Cleoceri, N.L. and S.F. Lee. (1965a).
Hydrolysis of condensed phosphate - I: Non-sterile environment.
Air and Water Pollution, 9, 723-742.

Cleoceri, N.L. and S.F. Lee.(1965b).
Hydrolysis of condensed phosphates - II: Sterile environment.
Air and Water Pollution, 9, 743-751.

Crowther, J.P. and A.E.R. Westman. (1954).
The hydrolysis of the condensed phosphates - I: Sodium pyrophosphate and sodium triphosphate.
Can. J. Chem., 32, 42-48.

Crowther, J.P. and A.E.R. Westman. (1956)
The hydrolysis of condensed phosphates - III: Sodium tetrametaphosphate and sodium tetraphosphate.
Can. J. Chem., 34, 969-981.

deMan, J.M. (1966).
Effect of ortho- and polyphosphates on milk stability as measured by rennet coagulation time.
XVII Intern. Dairy Congr. 17th proc., Munich B, p. 365.

deMan, J.M. (1967).
Determination of inorganic phosphorus.
Unpublished data.

Engelbrecht, R.S. and J.J. Morgan. (1959).
Studies on the occurrence and degradation of condensed phosphates in surface water.
Sewage and Industrial Wastes, 31, 458-478.

Friess, S.L. (1952).
The energetics of acid-catalyzed hydrolysis of triphosphoric and pyrophosphoric acids.
J. Am. Chem. Soc., 74, 4027-4029.

Froning, S.W. (1966).
Effect of various additives on the binding properties of chicken meat.
Poultry Science, 45, 185-188.

Gassner, K.C. (1958).
Uber die analytik bond ensierter phosphate in Lebensmitteln.
In Kondensierte Phosphate in Lebensmittel (a symposium), Springer Verlag, Berlin.

Gisske, W. (1958).
Effect of phosphate on quality of cooked ham.
Fleischwirtschaft, 10, 21-22.
Cited from J. Sci. Fd. Agr. Abstr. 239 (2), 1958.

Green, Jerome. (1950).
Reversion of molecularly dehydrated sodium phosphates.
Industrial and Engineering Chemistry, 42 (8), 1542-1546.

Grau, R. (1955).
Misunderstandings and misconceptions regarding phosphates.
Die Fleischwirtschaft, Jan., 7, 15-16.

Hamm, R. (1955).
The cause of the action on meat of additives in the mince and common salt.
Fleischwirtschaft, 7, 196-203.
Cited from Fd. Sci. Abstr., 27, 491.

Hamm, R. and R. Grau. (1955).
Uber die Wirkung von phosphaten auf die Wasserbindung des Fleisches.
Dtsch. Lebensmitt - Rdsch., 51, 106-111.
Cited from Fd. Sci. Abstr., 28, 18, 1956.

Harold, F.M. and Ruth L. Harold. (1965).
Degradation of inorganic polyphosphate in mutants of Aerobacter aerogenes.
Journal of Bacteriology, 89, 1262-1270.

Herrmann, H. and G. Perlmann. (1937).
Reaction between proteins and metaphosphoric acid.
Nature, 140, 807.

Holmquist, J.W., C.F. Schmidt, and A.E. Guest. (1948).
The use of hexametaphosphate in the blanching of peas.
Canning Trade, 70 (40), 7-8, 20.

Inklaar, P.A. (1967).
Interaction between polyphosphates and meat.
Journal of Food Science, 32 (5), 525-526.

Karl-Kroupa, Editha, Clayton F. Callis and Eli Seifter. (1957).
Stability of condensed phosphates in very dilute solutions.
Industrial and Engineering Chemistry, 49 (12)

Kiehl, Samuel J. and W.C. Hansen. (1926).
The effect of hydrogen-ion concentration upon the rate of hydration of sodium pyrophosphate. Phosphate II.
J. Am. Chem. Soc. 48, 2802-2814.

Kiehl, Samuel J. and Harry P. Coats. (1927).
The hydration of sodium monometaphosphate in alkaline solution at 75° Phosphate V.
J. Am. Chem. Soc. 49, 2180-2193.

Klose, A.A., A.A. Campbell and H.L. Hanson. (1963).
Influence of polyphosphates in chilling water on quality of poultry meat.
Poultry Science 42, 743-749.

Lehmann, B.T. and B.M. Watts. (1951).
Antioxidants in aqueous fat systems.
J. Am. Oil Chemist Soc. 28, 475.

Leviton, A. and M.J. Pallansch. (1962).
High-temperature short-time evaporated milk. IV. The retardation of gelation with condensed phosphate, manganous ions, polyhydric compounds and phosphatides.
J. Dairy Science, 45, 1045-1056.

Love, R.M. and G. Abel. (1966).
The effect of phosphate solutions on the denaturation of frozen cod muscle.
Journal of Food Technology, 1, 323-333.

Lyons, J.W. and C.D. Siebenthal. (1966).
On the binding of condensed phosphates by proteins.
Biochem. Biophys. Acta, 120, 174-176.

Marion, W.W. and R.N. Forsythe. (1962).
Protection afforded lipids of turkey meat by butylated hydroxy anisole, egg white solids, gelation and tripolyphosphate.
Poultry Science 41, 1663.

May, K.N., R.L. Helmer and R.L. Saffle. (1962).
Effect of phosphate treatment on carcass weight changes and organoleptic quality of cut-up chicken.
Poultry Science 41 (2), 1665. Abstracts.

McCullough, J.F., J.R. Van Wazer and E.J. Griffith. (1956).
Structure and properties of the condensed phosphates - XI: Hydrolytic degradation of Graham's salt.
J. Am. Chem. Soc. 78, 4528-4533.

McFee, E.P. and R.L. Swaine. (1953).
Stops struvite with new technique.
Food Engineering, 25, (2), 67, 190.

McGilvery, J.D. and J.P. Crowther. (1954).
The hydrolysis of the condensed phosphates - II: (A) The role of the hydrogen ion in the hydrolysis of sodium pyrophosphate; (B) The dissociation constants of pyrophosphoric acid.
Can. J. Chem. 32, 174-185.

Monk, J.A., G.J. Mountney and I.Prudent. (1964).
Effect of phosphate treatment and cooking method on moisture losses of poultry meat.
Food Technology, 18, 104-107.

Morse, R.E. (1955).
How phosphates can benefit meats.
Food Engineering, 2, 84-86.

Mountney, G.J. and F.C. Arganosa. (1962).
The effect of phosphates on moisture absorbance, retention and cooking losses of broiler carcasses.
Poultry Science 41 (2), 1668 Abstracts.

Odagiri, S. and T.A. Nickerson. (1964).
Chain length determination of polyphosphates.
J. Dairy Sci. 47, 920.

Perlmann, G. and H. Herrmann. (1938).
On the reaction between metaphosphoric acid and egg albumin.
Biochem. J. 32, 926-930.

Polley, J.R. (1949).
Microdetermination of inorganic phosphate in plasma and urine.
Can. j. Res. 27, 265.

Ramsey, M.B. and B.M. Watts. (1963).
The antioxidant effects of sodium tripolyphosphate and vegetable extracts on cooked meat and fish.
Food Technology 17, 1056-1059.

Roesler, H. (1966).
Verhalten der polyphosphate in Schmilkase.
Milchwissenschaft, 21 (2), 104-107.

Sawyer, C.N. (1952).
Some new aspects of phosphates in relation to lake fertilization.
Sewage and Industrial Wastes, 24, 768-776.

Schermerhorn, E.P., R.L. Adams and W.J. Stadelman. (1963).
Effects of polyphosphates on water uptake, moisture retention and cooking loss in broilers.
Poultry Science 42, 107-110.

Scharpf, L.G., Jr. and T.P. Kichline. (1967).
Stability of a long chain polyphosphate in aqueous cheese extracts.
J. Agr. Food Chem. 15 (5), 787-789.

Siewart, S. and J.G. Woodroof. (1952).
Unshelled peanuts now salted by new process.
Food Engineering 24 (11), 110-111.

Smith, M.J. (1959).
Independence in hydrolysis of condensed phosphates.
Can. J. Chem. 37, 1115-1116.

Spencer, J.V. and L.E. Smith. (1962).
The effect of chilling chicken fryers in a solution of polyphosphates upon moisture uptake, microbial spoilage, tenderness, juiciness and flavor.
Poultry Science 41 (2), 1685 Abstracts.

Swift, C.E. and Rex Ellis. (1956).
The action of phosphates in sausage products.
I. Factors affecting the water retention of phosphate treated ground meat.
Food Technology, 10, November, 546-552.

Tanikawa, E., M. Akiba and A. Shitamori. (1963).
Cold storage of cold fillets treated with polyphosphates.
Food Technology, 17, 1425-1429.

Thomson, J.E. (1964).
Effect of polyphosphates on oxidative deterioration of commercially cooked fryer chickens.
Food Technology, 18, 1805-1806.

Tims, Margaret J. and Betty M. Watts. (1958).
Protection of cooked meats with phosphates.
Food Technology, 12, 240-243.

Trumic, Z. and N. Masic. (1962).
Utilization of polyphosphates in canned ham production.
Technol. Mesa., 3 (12), 13-15.

Van Wazer, J.R. and Karl A. Holst. (1950).
Structure and properties of the condensed phosphates. I.
Some general considerations about phosphoric acid.
Journal of the American Chemical Society, 72, 639-644.

Van Wazer, J.R., E.J. Griffith and J.F. McCullough. (1952).
Hydrolysis of condensed phosphates.
J. Am. Chem. Soc. 74, 4977-4978.

Van Wazer, J.R., E.J. Griffith and J.F. McCullough. (1955).
Structure and properties of the condensed phosphates - VII:
Hydrolytic degradation of pyro- and tri-polyphosphate.
J. Am. Chem. Soc. 77, 287-291.

Van Wazer, J.R. (1958).
Phosphorus and its compounds.
Interscience Publishers Inc., New York, 1958, Vol. 1, Chapter 8, pp. 419-477.

Vasic, J. and J.M. deMan. (1966).
The composition of the liquid phase of milk fat obtained by a pressure filtration method.
XVII International Dairy Congress, München, 149-153.

Vujicic, I., S.C. Batra and J.M. deMan. (1967).
Interaction of alkaline earth metal ions with polyphosphates and citrate in the presence and absence of casein.
Journal of Agriculture and Food Chemistry, 15 (3), 403-407.

Wilson, H.K., J.L. Vetter, K. Sasago, and E.O. Herreid. (1963).
Effects of phosphates added to concentrated milks before sterilization at ultra-high temperatures.
J. Dairy Science 46, 1038-1043.

Yasui, T., M. Sakanishi and Y. Hashimoto. (1964).
Effect of inorganic polyphosphates on the solubility and extract ability of Myosin B.
Journal of Agricultural and Food Chemistry, 12 (5), 392-399.